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Original Paper

Effects of biochar and biochar with nitrogen on soil organic matter and soil structure in Haplic Luvisol

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An experiment of different application rates of biochar and biochar combined with nitrogen fertilizer was conducted at the newly established experimental field (spring 2014) on a Haplic Luvisol located in Nitra region of Slovakia during the growing season of spring barley. The aim of this study was to evaluate the effects of biochar combined with fertilization on the soil organic matter and soil structure parameters. The treatments (3 replicates) consisted of 0, 10 and 20 t ha^{-1} of biochar application (B0, B10 and B20) combined with 0, 40 and 80 kg ha^{-1} of nitrogen fertilizer applied (N0, N40, N80). The results showed that the effect of biochar application without N fertilization significantly decreased the easily extractable glomalin in B10N0 and B20N0 compared to B0N0, respectively. The same effects were observed in B10N40 and B10N80. The soil organic matter (SOM) was rapidly degradable by micro-organisms (on the base of lability index values) in B10N0 treatment and the SOM had greater stability and resistance to microbial degradation in B10N80 treatment. Added N fertilization in both doses together with 10 t biochar ha^{-1} had statistical significant influence on decreasing of lability index values. The highest accumulation of carbon occurred in B20N0 treatment. The addition of biochar at 10 t ha^{-1} together with 80 kg ha^{-1} N significantly increased values of carbon pool index (24%) compared to B10N0. Generally, the highest average content of macro-aggregates was found in the B20N0 treatment and then in B20N80 > B10N0 > B0N0 > B10N80 > B10N40 > B20N40. Treatment B10N0 showed robust increase (by 53%) for the macro-aggregates of > 7 mm, but on the other hand it decreased content of macro-aggregates 3–1 mm compared to B0N0. A considerable increase of aggregates stability was found in range of 19% in case of 20 t ha^{-1} of biochar application combined with 80 kg ha^{-1} N compared to B0N0. A positive effect on decrease of percentage of aggregate destruction was found only in case of B20N80 treatment compared to B0N0.

Keywords: biochar, N fertilization, carbon pool index, percentage of aggregate destruction, aggregate stability

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1 Introduction

Taking care of the soil is a demonstration of state development and the cultural level of its population. This knowledge about the soil combined with qualified state administration in its protection and land use have been one of the most important conditions of Slovakia's acceptance into the European Union. Nowadays, the growing problems related to the environmental quality of the cultivated land and long-term productivity of agro-ecosystems has a significant influence on the required development and improvement of management strategies that maintain and protect the soil functions. Despite the fact that the soil is considered a unique and irreplaceable natural resource worldwide, the reduction of its acreage for agricultural use is continually being recorded. The database of the Soil Science and Conservation Research Institute showed, that the average soil loss from the agricultural soil fund in the Slovak Republic was about 1000–5000 ha per year, representing about 3 to 14 hectares per day and it is expected that this decline will continue to increase. Therefore, the total and permanent soil loss must be addressed or compensated through the improvement of its parameters. In terms of sustainable management, it is very important to preserve or maintain favorable chemical, physical, physico-chemical and biological soil properties, which have very close bearing on the soil organic matter (SOM).

SOM represents a considerable pool of carbon with the turnover time from a year to tens of years (for vegetation residues) and from hundreds to thousands of years for soil humus (Schepaschenko et al., 2013). SOM plays an important role in maintaining soil quality and ecosystem functionality (Benbi et al., 2015) and it is an important aspect of agricultural soil quality and soil ecology (Gaida et al., 2013). SOM is a dynamic entity influenced by several factors, such as climate, clay content and mineralogy and soil management etc. (Loveland and Webb, 2003; Schepaschenko et al., 2013). The organic carbon content in the soil (*SOC*) is one of the qualitative parameters of the soil humus regime (Howard and Howard, 1990).

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One of the soil's most important physical properties is a soil structure which is important for many soil environmental processes (Garbout et al., 2013). It is a key factor in the functioning of the soil, its ability to support plant and animal life, and moderate environmental quality (Bronick and Lal, 2005). In particular, soil structure is one of the most important factors in the stabilization of the soil organic carbon, because this is a significant binding agent that associates of mineral particles together into aggregates (Chaplot and Cooper, 2015; Rabbi et al., 2015; Šimanský and Jonczak, 2016).

In general, agricultural soils, due to intensive use, have often outstanding balance of organic compounds leading to the *SOC* decline. The most important source of organic compounds are organic fertilizers especially farmyard manure. Since the last two decades has recorded the continual decline of livestock population in Slovakia, which also results in a decreasing production of organic fertilizers, it is extremely important to pay attention to equal balance of organic compounds in the arable soils. One such possible innovative solution may include the application of biochar, which is an important source of organic matter (Fischer and Glaser, 2012).

Biochar is the product of thermal decomposition of organic materials in the absence of air (pyrolysis), and is distinguished from charcoal by its use as a soil amendment (Lehmann, 2007; Lehmann and Joseph, 2009; Zimmerman, 2010). Its use in agriculture is justified because it has confirmed its positive effects on increasing yields mainly in sandy soils (Jeffery et al., 2011; Butnan et al., 2015), the improvement of chemical properties mainly increases of pH in acidic soils (van Zwieten et al., 2010; Horák, 2015) and improved nutrient regime of soils (Purakayastha et al., 2015). Biochar has also been shown to change soil biological community composition and abundance (Liang et al., 2010; Lehmann et al., 2011). Applied biochar to the soils has had positive effects on the physical properties of soils such as soil water holding capacity, bulk density, porosity (Hina et al., 2010; Kammann et al., 2011) large inner surface area (van Zwieten et al., 2009) and soil structure (Herath et al., 2013; Obia et al., 2016).

If the "modern" farmer wants to be successful he should not only be equipped with the considerable theoretical knowledge but also with innovative practical experiences from the discipline of soil management. Relationships between organic matter and soil structure have been studied in different climate conditions, soil types and soil managements (Elliot, 1986; Oades and Waters, 1991; Šimanský et al., 2013), but these relationships in the Haplic Luvisol which are most intensively used soils and especially after application of biochar in field conditions of Slovakia have not been clarified.

Under these contexts, we hypothesised that the application of biochar to the soil could increase SOM and improve the soil structure parameters. The objective of this study, therefore, was to determine whether the addition of biochar or biochar together with nitrogen fertilizer affects the soil organic matter and parameters of the soil structure.

2 Material and methods

2.1 Site description and experimental details

In 2014, the Department of Biometeorology and Hydrology of SAU Nitra established a field experiment in locality Dolná Malanta (lat. 48° 19′ 00″; lon. 18° 09 ′00″). The area is in a temperate climate and the average annual air temperature was 10.3 °C and annual precipitation was 640 mm during the studied year. The soil type is classified as the Haplic Luvisol (WRB, 2006). More information about the experimental base of SUA Nitra is published in Tobiašová and Šimanský (2009). Soil samples from 10 random locations (experimental field trial) were taken on 4th of March before setting up the experiment from soil depth of 0–20 cm. Soil carbon content was 9.13 g kg^{-1}, while the average soil pH (KCl) was 5.71. On average, soil contained 360.4 g kg^{-1} of sand, 488.3 g kg^{-1} of silt and 151.3 g kg^{-1} of clay (Šimanský et al., 2008).

Some days later the experiment was laid out (7th of March) followed by biochar application (10th of March) and sowing the crop (11th of March). The replicated (n = 3) trial plots (4 m × 6 m) were laid out in a randomized block design and planted with spring barley (*Hordeum vulgare* L.) in the experimental field that has been used for crop production over the last several years. The experiment consisted of following treatments separated by a protection row 0.5 m in width:

1. B0N0 – no biochar, no N fertilization
2. B10N0 – biochar (10 t ha^{-1})
3. B20N0 – biochar (20 t ha^{-1})
4. B10N40 – biochar (10 t ha^{-1}) + fertilizer (40 kg ha^{-1} N)
5. B20N40 – biochar (20 t ha^{-1}) + fertilizer (40 kg ha^{-1} N)
6. B10N80 – biochar (10 t ha^{-1}) + fertilizer (80 kg ha^{-1} N)
7. B20N80 – biochar (20 t ha^{-1}) + fertilizer (80 kg ha^{-1} N)

The field was ploughed, harrowed and biochar was evenly spread onto the soil surface and immediately incorporated into the soil (10 cm) combined with or without N fertilization. To maintain consistency, plowing and mixing treatments were also performed for the plots without biochar or N fertilization. Biochar used for the field experiment was produced from paper fiber sludge and grain husks (1 : 1 w/w) (company Sonnenerde, Austria) by pyrolysis at 550 °C for 30 minutes in a Pyreg reactor (Pyreg GmbH, Dörth, Germany). Biochar particle sizes ranged from 1 to 5 mm and on average it contained 53.1 g kg^{-1} of total carbon, 14 g kg^{-1} of total nitrogen and % of ash was 38.3. On average, the pH in KCl was 8.8. Used N fertilizer was nitre ammonium with dolomite (LAV 27).

2.2 Soil sampling and analytical methods

The soil samples were taken from soil depth of 0–20 cm during the whole spring barley growing season (19 March, 17 April, 15 May, 16 June, and 13 July). Three different locations at each treatment were chosen for soil sampling with samples being mixed to produce an average representative sample.

The determination of the particle-size distribution using the pipette method (Hrivňáková et al., 2011) consisted of the following procedure in the soil samples: dissolution of $CaCO_3$ with 2 M HCl, decomposition of the organic matter with 6% H_2O_2, repeated washing, dispersing using $Na(PO_3)_6$ and then determination of the particle-size distribution. The easily extractable glomalin related soil proteins (EEGRSP) was extracted from soil samples according to method established by Wright (Wright et al., 2006; Wright and Upadhyaya, 1996). Soil extraction includes autoclaving of weighed sample in a solution of sodium citrate. Briefly, the extraction process consists of following steps: weighing ca 1 g of soil and placing it in the 50 ml PP centrifuge tube, adding 20 mM sodium citrate (pH 7.0), autoclaving ($t = 121$ °C, $p = 1.4$ MPa), centrifuging and decanting of the supernatant (Wojewódzki and Cieścińska, 2012). The EEGRSP content was analyzed with utilization of Bio-Rad protein dye reagent (Bio-Rad 500–0006), which basic component is Coomassie brilliant blue used in the original Bradford protocol (Bradford, 1976). Extract's absorbance was measured at 595 nm by UV-VIS Smartspec spectrophotometer (Bio-Rad 170-2525). Disposable 50 μl cuvettes were used. Calibration curve was prepared based on ready solutions (Bio-Rad 500-0207) of bovine serum albumin (BSA) according to producer's protocol. When necessary, extracts were diluted by phosphate buffer saline (PBS) pH 7.4 (AppliChem A9177,0100). The soil organic carbon (*SOC*) content was assessed by the Tyurin Method of wet oxidation (Dziadowiec and Gonet, 1999). The labile carbon content (C_L) was assessed by Loginov Method (Łoginow et al., 1987). On the base of determined *SOC* and C_L the following parameters of SOM were calculated: lability index (*LI*), carbon pool index (*CPI*) and the carbon management index (*CMI*), as suggested Blair et al. (1995).

The carbon management index (*CMI*) was calculated according to the following equation:

$$CMI = CPI \times LI \times 100 \tag{1}$$

where:
CPI – the carbon pool index and *LI* is the lability index.

CPI and *LI* are calculated as follows:

$$CPI = \frac{C\ pool\ in\ treatment}{C\ pool\ in\ reference} \tag{2}$$

$$LI = \frac{Lc\ in\ treatment}{Lc\ in\ reference} \tag{3}$$

where:
L_C refers to the *C* lability, calculated as:

$$L_C = \frac{C_L}{C_{NL}} \tag{4}$$

and non-labile carbon (C_{NL}) is calculated as:

$$C_{NL} = SOC - C_L \tag{5}$$

where:
SOC – organic carbon content and C_L is labile carbon content

The control treatment (B0N0) was used as the reference and different biochar application rates combined without or with different N fertilizer levels as the treatment.

To determination of individual size fraction of aggregates, the AS 200 device (Retsch®) was used. The analysis began with 250 g sample of aggregates. Sieving was done with six sieves with mesh of 7, 5, 3.15, 1, 0.5 and 0.25 mm. The remaining material except for micro-aggregates (<0.25 mm) was quantified in each sieve. The micro-aggregate fraction calculated as the difference between the total weight of the soil sample and the sums of macro-aggregates (>0.25 mm). Size fractions of water-stable aggregates (*WSA*) for determination of indexes of aggregate stability (*Sw*) were determined using the Baksheev method (Vadjunina and Korchagina, 1986).

The indexes of aggregate stability (*Sw*), the percentage of aggregate destruction (*PAD*) and coefficient stability (K_S) were calculated according to following equations (6–8):

$$Sw = \frac{WSA - 0.09\,sand}{silt + clay} \tag{6}$$

where:
WSA – content of water-stable aggregates (%)

$$PAD = \frac{m_d - m_w}{m_d} \times 100 \tag{7}$$

where:
m_d – mass fraction of aggregates >0.25 mm after dry sieving
m_w – mass fraction of aggregates >0.25 mm after wet sieving

$$Ks = \frac{A}{B} \tag{8}$$

where:

A – the weight of *WSA* in size fractions from 0.25 to 10 mm

B – the weight of *WSA* less than 0.25 mm

Statistical analysis

Statistical analysis was performed using the Statgraphics Centurion XV.I programme (Statpoint Technologies, Inc., USA). For each soil used, the effects of biochar and biochar combined with N fertilizer on the SOM and soil structure parameters were tested using one-way ANOVA and then the least significant difference (LSD) method was used to compare treatment means for the two levels of biochar and two levels of nitrogen-treatments at the significant level of $\alpha = 0.05$.

3 Results

3.1 Effects of biochar on the soil organic matter

The effect of biochar application without N fertilization significantly decreased the easily extractable glomalin related soil protein content (EEGRSP) from 0.617 g kg^{-1} to 0.484 and 0.510 g kg^{-1} in B10N0 and B20N0, compared to control (B0N0), respectively. The same effects (decrease of EEGRSP) were observed when biochar was applied to the soil at rate of 10 t ha^{-1} together with 40 and 80 kg ha^{-1} N. Added N fertilizer in all biochar treatments did not have significant influence on EEGRSP values compared to biochar treatments without N fertilization.

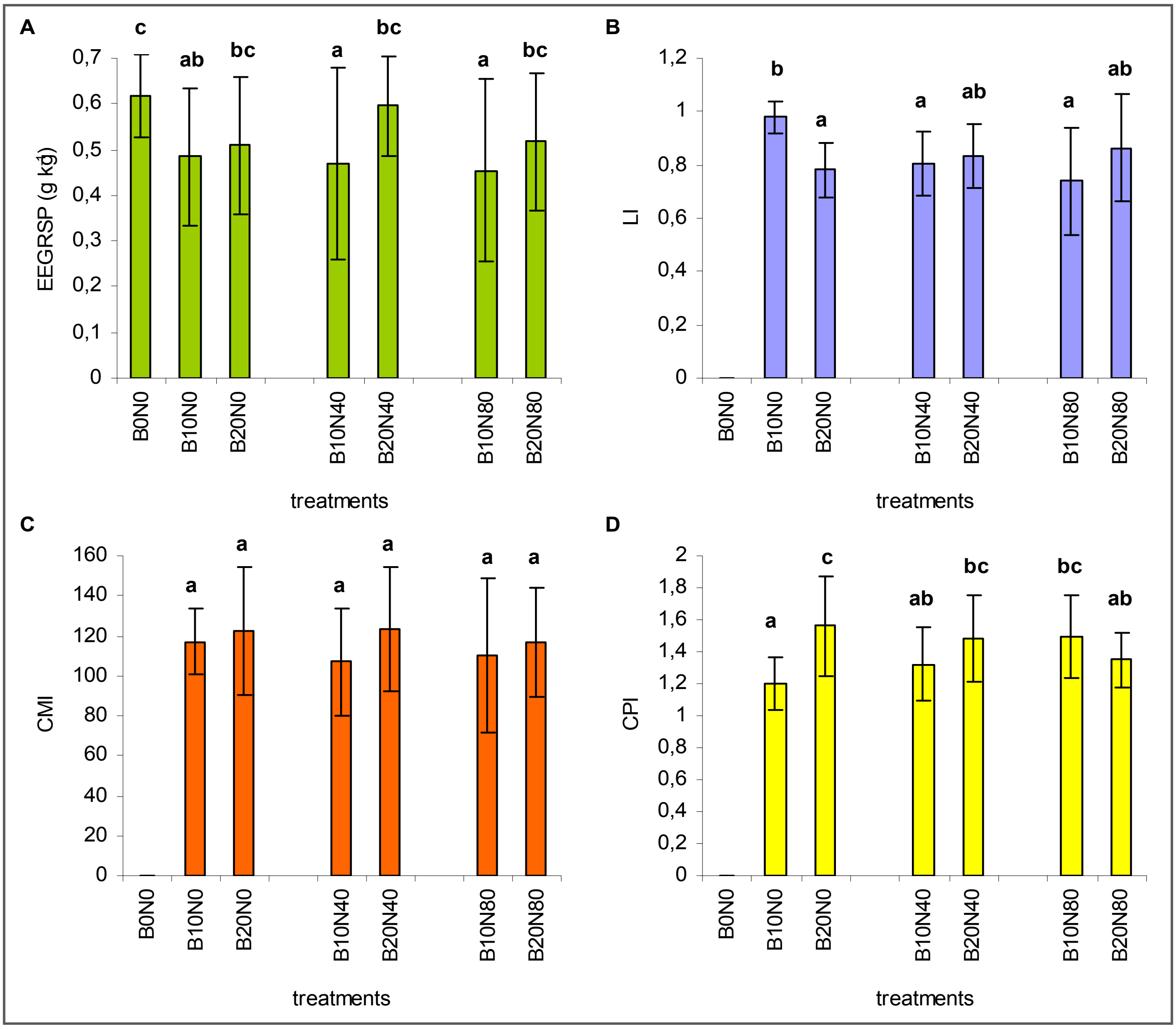

Figure 1 Statistical evaluation of A) easily extractable glomalin related soil protein content, B) lability index, C) carbon management index, and D) carbon pool index
Different letters between columns (a, b, c) indicate that treatment means are significantly different at $P < 0.05$ according to LSD multiple-range test

The SOM was rapidly degradable by micro-organisms (on the base of *LI*) in B10N0 treatment and the SOM had greater stability and resistance to microbial degradation in B10N80 treatment. Added N fertilization in both doses together with 10 t biochar ha^{-1} had statistical significant influence on decreasing of *LI* values. In B10N40 and in B10N80, the values of *LI* decreased by 18% and 25% respectively compared to B10N0. It means that higher dose of N fertilizer (80 kg ha^{-1}) resulted in higher decrease of *LI* values than lower dose of N. The lower the *CPI* value is, the more soil degradation is intensified in terms of reduction of soil organic matter content. Reviewing *CPI* indices, the highest accumulation of carbon occurred in B20N0 treatment. The effects of biochar with different levels of N fertilization on the *CPI* were evaluated, as well (Figure 1D). The addition of biochar at 10 t ha^{-1} together with 80 kg ha^{-1} N significantly increased values of *CPI* (24%) compared to B10N0. On the other hand there was found to be a considerable decrease of *CMI* (15%) due to the application of biochar at 20 t ha^{-1} combined with 80 kg ha^{-1} N compared to B20N0 treatment. Comparison of biochar addition and the lowest N fertilization level showed no effect on *CMI* (Figure 1C). The values of *CMI* in the soil to examine the impact of soil management practices were also calculated. When considering *CMI* indices, the most intense change was caused as a result of application of biochar at rate of 20 t ha^{-1} with 40 kg ha^{-1} N. Soil *CMI* decreased (no significant) in the following order: B20N40 > B20N0 > B10N0 > B20N80 > B10N80 > B10N40.

3.2 Effects of biochar on the soil structure parameters

Table 1 summarizes the application effects of biochar and biochar combined with nitrogen fertilization on the soil structure parameters. One-way ANOVA analysis showed the significant differences between treatments, for contents of micro-aggregates, but only for B20N0, B20N80 and B20N40 treatments. Generally, the highest average content of macro-aggregates was found in the B20N0 treatment and then in B20N80 > B10N0 > B0N0 > B10N80 > B10N40 > B20N40. The effects of biochar and biochar with different levels of N fertilization on the individual size fractions of macro-aggregates are shown in Figure 2. Treatment B10N0 showed robust increase (by 53%) for the macro-aggregates of >7 mm, but on the other hand it decreased content of macro-aggregates 3–1 mm compared to B0N0 (Figure 2). Application of only biochar at 20 t ha^{-1} had no noteworthy influence on content of macro-aggregates. A combination of biochar at 10 t ha^{-1} with lower level of N fertilization showed also significant increase in content of macro-aggregates >7 mm compared to B0N0. In this case, the effect of N fertilization was not significant. In this treatment (B10N40), the values of macro-aggregates in size fractions 5–3 mm (by 22%) and 3–1 mm (by 20%) were significant lower than in B0N0 treatment. The differences in contents of macro-aggregates at size fractions 5–1 mm between B10N40 and B0N40 were not observed. The treatment B10N40 had negligible effect on macro-aggregates (Figure 2). The treatment where biochar was combined with the highest level of N fertilization showed the most significant influence on the contents of macro-aggregates at the size fractions 7–5 mm as well as 3–1 mm. In B20N80, the values of macro-aggregates in size fractions 7–5 mm (27%) were higher and in the size fraction of 3–1 (13%), 1–0.5 mm (24%) and 0.5–0.25 mm (21%) were lower than in B0N0 treatment. Again, the values of macro-aggregates in size fractions 7–5 mm were also significantly higher by 27% and in the size fraction of 3–1 mm significantly lower by 13% in case of B10N80 as compared to B0N0

Table 1 Statistical evaluation of soil structure parameters

	Micro-aggregates	*Sw*	K_S	*PAD*
Treatments				
B0N0	10.1±0.74^{ab}	1.04±0.08^{a}	2.40±0.64^{a}	22.4±5.91^{b}
B10N0	10.0±1.22^{ab}	1.19±0.15^{ab}	4.78±2.44^{bc}	11.7±3.99^{ab}
B20N0	9.44±0.87^{a}	1.10±0.19^{ab}	4.33±1.70^{bc}	19.1±3.40^{ab}
B10N40	11.6±1.35^{ab}	1.04±0.13^{a}	2.54±1.21^{ab}	21.3±4.44^{ab}
B20N40	12.3±3.59^{b}	1.14±0.18^{ab}	4.20±2.81^{bc}	12.7±3.40^{ab}
B10N80	10.7±1.58^{ab}	1.08±0.13^{ab}	2.90±1.02^{ab}	18.7±4.35^{ab}
B20N80	9.90±1.81^{a}	1.24±0.08^{b}	5.13±1.44^{c}	8.22±2.47^{a}

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

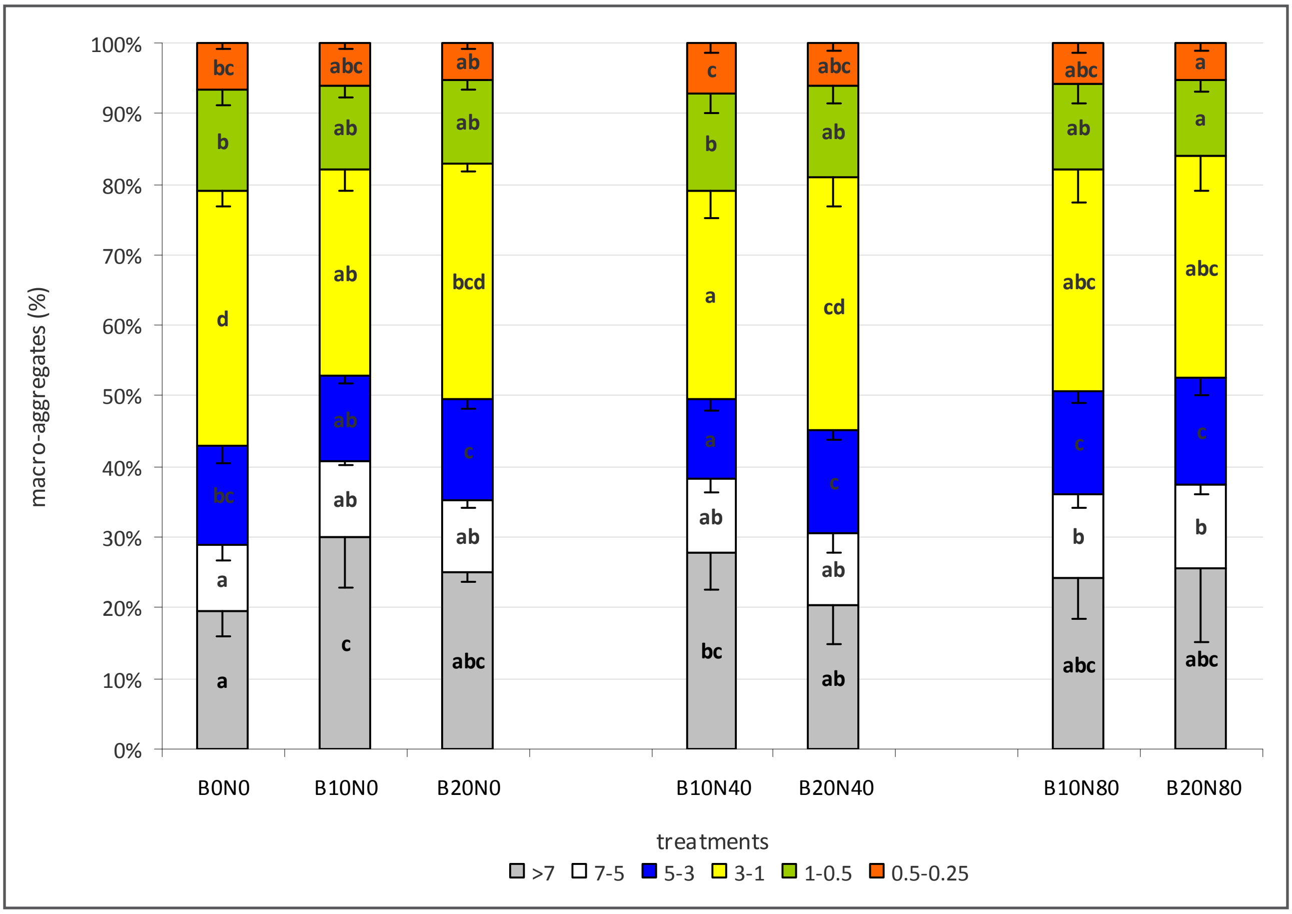

Figure 2 Statistical evaluations of individual size fractions of macro-aggregates
Different letters between columns (a, b, c, d) indicate that treatment means are significantly different at *P* <0.05 according to LSD multiple-range test

treatment. The effect of N fertilization on content of macro-aggregates was confirmed only in case of B10N80 treatment. There content of macro-aggregates in size fraction 5–3 mm increased by 20% compared to B10N0.

Other parameters of the soil structure were also evaluated (Table 1). The stability of the aggregates (*Sw*) was not increased due to different rates of biochar application to the soil compared to control soil (B0N0). Similarly, the application of 10 and 20 t ha^{-1} of biochar together with 40 kg ha^{-1} N had no effect on the aggregates stability compared to B0N0, B10N0 and B20N0 treatments. A considerable increase of aggregates stability was found in range of 19% in case of 20 t ha^{-1} of biochar application combined with 80 kg ha^{-1} N compared to B0N0, however, between B20N80 and B20N0 treatments any significant difference was not observed. The values of the percentage of aggregate destruction (*PAD*) had the reverse impact of the *Sw* values (Table 1). Overall, the highest average values of *PAD* were found when no biochar was applied in the unfertilized treatment (B0N0). A positive effect on decrease of *PAD* was found only in case of B20N80 treatment compared to B0N0. Generally, the biochar increased the coefficient aggregate stability (Ks) at all fertilized treatments. The increase in Ks was 99%, 80%, 75% and 114% for the B10N0, B20N0, B20N40 and B20N80 respectively compared to B0N0. If the biochar was combined with 40 and 80 kg N ha^{-1}, the N fertilization had negligible effect on Ks values (Table 1).

4 Discussion

4.1 *Effects of biochar on the soil organic matter*

As it is well known the higher glomalin concentration is beneficial for the soil due to forming soil aggregation, C accumulation and reducing erosion (Rillig and Steinberg, 2002; Wu et al., 2014) therefore the effects of biochar and biochar combined with N fertilization on glomalin concentration was evaluated (Figure 1A). The effect of biochar application without N fertilization significantly decreased the easily extractable glomalin (EEGRSP) in B10N0 and B20N0, compared to B0N0, respectively. The same effects were observed in B10N40 and B10N80. The chemical changes including higher loading of chemical fertilizer have negative effect on glomalin concentration (Alguacil et al., 2014; Wu et al., 2014). With higher content of *SOC* connected the increase of glomalin concentration (Fokom et al., 2012). This relationship was not confirmed in this and previous studies (Šimanský et al. 2016; Šimanský et al., 2017). Several studies (Fisher and Glasher, 2012; Mekuria and Noble, 2013) supported the fact about the positive effects of biochar application on increase of soil organic carbon. The same trends (increase of *SOC*) were observed in treatments when biochar was applied with N (40 and 80 kg ha^{-1} N), however, added N had a different effect on the *SOC* (Šimanský et al., 2016). Results of Šimanský et al. (2016) also showed that the C_L contents increased due to the application of biochar. However, these effects were not observed in all N treatments. The effects of biochar and biochar with N on changes in SOM parameters such as: *LI*, *CPI* and *CMI*, which are used for determination of smaller changes and changes over a short time period (Szombathová, 1999; Šimanský and Zaujec, 2009; Bendi et al., 2015) were evaluated in this study (Figures 1 B, C, D). Higher doses of biochar with no N fertilization and lower doses of biochar applied with higher doses of N appear to increase SOM resistance to microbial degradation and on the other hand, the SOM was rapidly degradable by micro-organisms in B10N0 treatment. This can be linked to the priming effect as reported by Fisher and Glaser (2012). Biochar could cause a positive priming effect due to its high surface area providing habitat for micro-organisms and due to input of partly labile C substrate (condensates). On the other hand, biochar is a stable compound, which could stabilize labile compost organic matter thus providing a negative priming effect. Using Conteh et al. (1999) recommendation of the use of *CPI* for determination of SOM content, we found that the lower the *CPI* value, the more soil degradation is intensified in terms of reduction of the soil organic matter content. The lowest values of the *CPI* were detected as a result of 10 t ha^{-1} of biochar application. Several authors (Šimanský and Zaujec, 2009; Šimanský and Polláková, 2012, 2016; Vieira, 2007; Benbi et al., 2015) reported that the soil management practices mainly fertilization has effect on *CPI* values. The results of this study also showed that the highest accumulation of carbon, as well as decomposable organic matter occurred (*CMI*) when 20 t ha^{-1} of biochar was applied without or with 40 kg ha^{-1} N. Higher doses of N together with biochar at rates of 10 and 20 t ha^{-1} as well as lower doses of N fertilization combined with 10 t biochar ha^{-1} decreased accumulation of SOM more rapidly, but no significant than the lower dose of N combined with higher dose of biochar, which confirmed the findings of several studies (Shimizu et al., 2009; Yang et al., 2011).

4.2 Effects of biochar on the soil tructure parameters

Several authors (Atkinson et al., 2010; Herath et al., 2013) confirmed the positive effect of biochar on soil structure. In Table 1 are showed the significant differences between treatments, for contents of micro-aggregates, but only for B20N0, B20N80 and B20N40 treatments. On the other hand, the highest average content of macro-aggregates was found in the B20N0 treatment and then in B20N80 and the lowest was determined in B20N40 treatment. Differences in the individual fractions of macro-aggregates due to biochar and biochar combined with N fertilization were also observed (Figure 2). These effects are probably influenced by the amount of biochar applied into the soil. The lower amounts of biochar are more suitable to become the part of the aggregates than higher ones. Secondly, biochar particles are 1–5 mm large and therefore difficult to become part of lower macro- and micro-aggregates. Most of all, the biochar is very stable in the soil compared to the other organic matter additions (Fischer and Glaser, 2012), which might be the reason why the solo application at a high dose did not appear to significantly influence the macro-aggregates content. Only application of N fertilizer in dose of 80 kg ha^{-1} together with 10 t biochar ha^{-1} had a positive effect on building-up of biochar into the aggregates in size fractions 5–3 mm. In other treatment any statistical significant changes were not observed. Added nitrogen to the soil can improve the processes of biochar mineralization and the result can be also a higher aggregation (Bronic and Lal, 2005). Biochar contributes to the formation of micro-aggregates (Brodowski et al., 2006) which was not confirmed in this study (Table 1). There are reported studies about the positive effects on the aggregate stability (Herath et al., 2013; Soinne et al., 2014). This study does not fully confirm these findings (Table 1). The stability of the aggregates was not increased due to biochar or biochar together with N fertilizer application compared to unfertilized soil (B0N0), however, in case of application 20 t biochar ha^{-1} combined with 80 kg N ha^{-1} it was otherwise. There was found to be a sizeable increase of aggregates stability in range of 19% in the B20N80 treatment compared to B0N0 treatment. The reasons for higher aggregate stability could be explained by the application of higher doses of biochar together with nitrogen. Fertilizer applications generally improve soil aggregation (Munkholm et al., 2002). The effect of improved nutrient management lead to increasing biomass and enhanced root growth (Abiven et al., 2015) also leading to increased root activity. Root activity, together with the direct effect of biochar acting as a binding agent of soil particles (Brodowski et al., 2006) could be responsible for the increase in aggregate stability. The higher root biomass through exudates and moving soil particles help aggregate formation (Bronick and Lal, 2005) and it was probably also the reason for higher aggregate stability in N80B20 treatment (Table 1). The results in this study also confirmed that applied biochar at rate of 20 t ha^{-1} together with 80 kg ha^{-1} N had a significant influence on the decrease of *PAD* values. Biochar after mineralization can flocculate together with soil particles and the result of this process is better structural state (Cheng et al., 2006; Jien and Wang, 2013).

5 Conclusions

Higher doses of biochar with no N fertilization and lower doses of biochar applied with higher doses of N appear to increase SOM resistance to microbial degradation and on the other hand, the SOM was rapidly degradable by micro-organisms in case of 10 t ha^{-1} of biochar application without N fertilization. The highest accumulation of carbon occurred in treatment with applied biochar in dose of 20 t ha^{-1}. A considerable increase of aggregates stability as well as a positive effects on decrease of the percentage of aggregate destruction were found in case of 20 t ha^{-1} of biochar application combined with 80 kg ha^{-1} N.

The results of this study demonstrated that biochar can improve the SOM and the physical condition (soil structure parameters) of the Haplic Luvisol. Finally, the results of this study also indicate that the application of biochar can be potential innovative method for sustainable soil management in arable soils of Slovakia.

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