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How dose of biochar and biochar with nitrogen can improve the parameters of soil organic matter and soil structure?

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Running Title: Soil organic matter and soil structure

Abstract: Biochar application to agricultural soils has a significant potential to influence soil resource availability and thus crop performance. A factorial experiment investigating effects of different biochar application rates combined with nitrogen fertilizer was conducted in field conditions on Haplic Luvisol. The aim of this study was to evaluate the effects of biochar and biochar combined with fertilization on soil organic matter and soil structure parameters. The treatments comprised combinations of biochar application of 0, 10 and 20 t ha^{-1} (B0, B10 and B20) and 0, 40 and 80 kg N ha^{-1} of nitrogen fertilizer (N0, N40, N80) applied in a full-factorial design. Biochar application rate of 20 t ha^{-1} significantly increased soil organic carbon content (*SOC*) and non-labile carbon content (C_{NL}), but decreased carbon lability (L_C). The addition of biochar at 10 t ha^{-1} together with 40 and 80 kg N ha^{-1} significantly increased the values of *SOC* and C_{NL}. On the other hand, B10N80 treatment resulted in a considerable decrease of carbon lability (L_C). Overall, the highest average content of water-stable macro-aggregates was found in the B20N80 treatment and then with B10N0 < B20N40 < B20N0 < B10N80 < B0N0 < B10N40. Biochar applied at 20 t ha^{-1} increased the critical level of soil organic matter and decreased the crusting index.

Key words: Biochar; N fertilization; Soil organic matter; Soil structure; Water-stable aggregates.

Introduction

The growing need for soil management strategies that enhance wider environmental benefits derived from arable soils while maintaining their productive function requires the development of novel approaches which ensure long-term sustainability of crop production. It is crucial to preserve or enhance favorable chemical, physical and biological soil properties, which in most soils are closely correlated with soil organic matter (*SOM*) content. *SOM* plays an important role in maintaining soil quality and ecosystem functionality (Benbi et al. 2015) and is an important aspect of agricultural soil quality and soil ecology (Gaida et al. 2013). Soil organic carbon (*SOC*) content is one of the qualitative parameters of the soil humus regime (Howard & Howard 1990). Soil structure is one of the soil's most important physical properties, critically important for many soil environmental processes (Czachor & Lichner 2013; Garbout et al. 2013; Kodešová et al. 2015; Leelamanie & Mapa 2015). Soil structure is the key factor regulating soil function, its ability to support plant and animal life, and moderate environmental quality (Bronick & Lal 2005).

Intensive agriculture often leads to a decline of *SOC* content. Organic fertilizers, such as farmyard manure, are often the most important sources of organic compounds in systems with continuous removal of organic crop residue. The last two decades have been characterized by a continuous decline of livestock population in Slovakia, resulting in a decreasing availability of organic fertilizers. At the same time, environmental and regulatory constraints have driven arable agriculture towards lower-input soil management, highlighting the need to maintain optimal soil function and a favourable balance of organic compounds in the soil. could be an Application of biochar to arable soils could constitute an alternative to the historical use of organic fertilizers, acting as an important source of stable organic matter (Lehman 2007; Fischer & 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 (Zimmerman 2010). Its use in agriculture may be advisable due to the confirmed positive effects on crop yields, mainly in sandy soils (Butnan et al. 2015). Biochar amends soil chemical properties such as *pH* (van Zwieten et al. 2010), reduces pesticide and nutrient leaching to groundwater (Novak et al. 2009), and improves nutrient regime of soils (Purakayastha et al. 2015). Biochar has also been shown to change soil biological community composition and abundance (Lehmann et al. 2011). Applying biochar to soils has had positive effects on soil physical properties, such as soil water holding capacity, bulk density, porosity

(Kammann et al. 2011) inner surface area (van Zwieten et al. 2009) and soil structure (Obia et al. 2016).

The relationship between organic matter and soil structure have been studied previously in different soil types, climate conditions and under varying soil management practices (Leelamanie & Karube 2014; Bartlová et al., 2015; Rajkai et al. 2015; Schacht & Marschner 2015). However, the interaction between biochar and biochar with nitrogen fertilizer applied in commercial setting and in field conditions has not been explored yet.

In this context, we hypothesised that the application of biochar to the soil would (i) increase *SOM* and (ii) improve soil structure. The objective of this study was to determine whether the addition of biochar or biochar together with nitrogen fertilizer has an effect on the soil organic matter and parameters of the soil structure.

Material and methods

Site description and experimental details

The field trial was conducted at an experimental site of SAU-Nitra (Nitra-Malanta) in Nitra region of Slovakia (lat. 48°19´00´´; lon. 18°09´00´´) during the period from March to July 2014 when a single crop of spring barley was grown. The soil at the site is classified as Haplic Luvisol (WRB 2006). Average annual air temperature was 10.3°C and annual precipitation was 640 mm during 2014. Soil samples from soil depth of 0–20 cm at 10 random locations (experimental field trial) were taken on 4th of March prior to setting up the experiment. On average, the soil contained 360.4 g kg^{-1} of sand, 488.3 g kg^{-1} of silt and 151.3 g kg^{-1} of clay. Soil organic carbon content was 9.13 g kg^{-1}, while the average soil *pH* (KCl) was 5.71.

The experiment was established on 7th March 2014, followed by biochar application (10th of March) and crop drilling (11th of March). The replicated (n=3) spring barley (*Hordeum vulgare L.)* trial plots (4 m x 6 m) were laid out in a randomized block design in an experimental field that has been used for continuous crop production for the last several years. The experiment consisted of the following treatments, separated by a protection row 0.5 m in width (Fig. 1): 1. B0N0 - no biochar, no N fertilization, 2. B10N0 - biochar (10 t ha^{-1}), 3. B20N0 - biochar (20 t ha^{-1}), 4. B0N40 - no biochar, fertilizer (40 kg N ha^{-1}), 5. B10N40 - biochar (10 t ha^{-1}) + fertilizer (40 kg N ha^{-1}), 6. B20N40 - biochar (20 t ha^{-1}) + fertilizer (40 kg N ha^{-1}), 7. B0N80 - no biochar, fertilizer (80 kg N ha^{-1}), 8. B10N80 - biochar (10 t ha^{-1}) + fertilizer (80 kg N ha^{-1}), 9. B20N80 - biochar (20 t ha^{-1}) + fertilizer (80 kg N ha^{-1}).

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 fertilization using a combinator. To maintain consistency, plowing and mixing treatments were also performed in the control plots where no biochar or N fertilization was applied. A standard N fertilizer (Calc-Ammonium nitrate with dolomite, LAD 27) was used in this experiment. Biochar 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) with particle size of 1–5 mm in size when applied (Table 1).

Soil sampling and analytical methods

Soil samples were repeatedly taken from the soil depth of 0–20 cm to cover the whole spring barley growing season (19th March, 17 th April, 15 th May, 16 th Jun, and 13 th July). Three different locations within each replicate plot were selected for soil sampling with and samples from a single plot were pooled to produce an average representative sample. Soil samples were always collected from the vicinity of the same sampling points and taken with the aid of a spade to maintain the soil aggregates throughout the experiment. Any roots and large fragments of plant litter were removed. Soil samples were transported to the laboratory where the large clods were gently broken up along natural fracture lines, followed by air-drying at the lab temperature.

Particle-size distribution was determined by the pipette method (Fiala et al. 1999): 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. Soil organic carbon content (*SOC*) was estimated by the Tyurin wet oxidation method. The reagent mixture used in 0.07 M H_2SO_4 and $K_2Cr_2O_7$, with titration using 0.01M Mohr's salt (Dziadowiec & Gonet 1999). Labile carbon was extracted from 1 g soil samples by shaking them in 50 mL of 0.005 M $KMnO_4$ for two hours. After centrifugation, labile carbon content (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 (Loginow et al. 1987). On the base of determined *SOC* and C_L we calculated the following parameters of *SOM*: carbon lability (L_C) and non-labile carbon content (C_{NL}), as suggested by Blair et al. (1995).

The L_C was calculated according to equation (1):

$$L_C = \frac{C_L}{C_{NL}} \tag{1}$$

where the non-labile carbon content (C_{NL}) is calculated as:

$$C_{NL} = SOC - C_L \tag{2}$$

Size classes of water-stable aggregates (*WSA*) were determined using the Baksheev method (Vadjunina & Korchagina 1986). Soil samples were first overflowed with distilled water (water level 1 cm above aggregates). After two hours, each sample was transferred to the top sieve (>5 mm) of a cylindrical container (Baksheev device), which was filled with distilled water. The cylinder was hermetically sealed and the sample was sieved for 12 minutes. The size fractions of *WSA* were as follows: >5, 5–3, 3–2, 2–1, 1–0.5, 0.5–0.25 mm (macro-aggregates, WSA_{ma}) and <0.25 mm (micro-aggregates, WSA_{mi}). The remaining material except for WSA_{mi} was quantified in each sieve. The micro-aggregate fraction was calculated as the difference between the total weight of the soil sample and the sums of macro-aggregates. The index of crusting (Lal & Shukla 2004), and the critical level of soil organic matter according to Pieri (1991) were calculated as well.

The index of crusting (*Ic*) was calculated according to equation (3):

$$Ic = \frac{1.5S_f + 0.75S_c}{Cl + (10xSOM)} \tag{3}$$

where S_f is % fine silt, S_c is % coarse silt, *Cl* is % clay, and *SOM* is % soil organic matter content.

Critical soil organic matter content (*St*) was calculated using equation (4):

$$St = \frac{SOM}{(Clay + Silt)} \tag{4}$$

Statistical analysis

Statistical analysis was performed using the Statgraphics Centurion XV.I programme (Statpoint Technologies, Inc., USA). Effects of biochar and biochar combined with N fertilizer on *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 application at the significant level of $\alpha = 0.05$. The link between the *SOM* and soil structure parameters was assessed by a correlation matrix.

Results and discussion

Effects of biochar on the soil organic matter

SOC content was higher in B10N0 (14.5±1.34 g kg^{-1}) and B20N0 (18.9±3.30 g kg^{-1}) than in B0N0 (12.2±0.85 g kg^{-1}). Biochar addition has been shown to increase *SOC* in soils (Fisher & Glasher 2012; Agegnehu et al. 2016). The same trends were observed in other treatments when biochar was applied with N (40 and 80 kg N ha^{-1}), however, N addition interacted with biochar addition effects on *SOC* (Table 2). Addition of N at 40 and 80 kg ha^{-1} together with 10 t ha^{-1} of biochar significantly increased the value of *SOC* by 10 and 24%, respectively, compared to B10N0. The C_{NL} contents reflected the *SOC* status. Overall, the highest average content of C_{NL} was found in the B20N0 and B10N80 treatments compared to B0N0 (Table 2). We also evaluated the effects of biochar and biochar with N on changes of L_C which is used for the determination of smaller changes and changes over a short period of time (Blair et al. 1995, Bendi et al. 2015). Higher values of L_C indicate that the *SOM* is readily degradable by micro-organisms, while lower values of L_C indicate that the *SOM* has greater stability and resistance to microbial degradation (Szobathová 1999, Laik et al. 2009). The values of carbon lability (L_C) were significantly affected in B20N0 and B10N80 treatments compared to B0N0. Higher doses of biochar with no N fertiziation and lower doses of biochar applied with higher doses of N appear to increase *SOM* resistance to microbial degradation.

Effects of biochar on the soil structure parameters

Several authors (Cornelissen et al. 2013; Herath et al. 2013) indicate positive effects of biochar on soil physical properties such as bulk density, porosity and soil structure stability. Our results from biochar-only plots are in general agreement with existing literature, but our study indicates the presence of interactive effects of biochar and nitrogen fertilization (Table 2). Biochar can improve soil physical conditions (Cornelissen et al. 2013; Obia et al. 2016) such as adsorption of cations (Liang et al. 2006), soil aggregate stability (Obia et al. 2016), but the effects on individual fractions of aggregates can differ, as indicated in our study (Fig. 2). For example, biochar (10 t ha^{-1}) applied without N fertilizer increased WSA_{ma} 5–2 mm content, but at the same time decreased WSA_{ma} 0.5–0.25 mm content. Application of biochar (20 t ha^{-1}) had no remarkable influence on the content of WSA_{ma}. Adding lower amounts of biochar may thus be more beneficial for soil aggregation than higher rates of biochar addition. Secondly, biochar particles are 1–5 mm in size when applied. Conversion to WSA_{ma} 0.5–0.25 mm might therefore be difficult and could occur only after some time. Most of all, the biochar is very stable in the soil compared to the other forms of organic matter applications (Fischer & Glaser 2012). The surface of biochar particles after oxidation may contain hydroxyl and carboxylic groups which are able to adsorb soil particles and clays and form macro-aggregates (Jien & Wang 2013) however this process requires a substantial lenght of time. In our case, the experiment was established in March 2014 and soil sampling was carried out during the same growing season. Biochar, due to its mostly inert nature, is often applied to soils in conjunction with organic or mineral fertilizers (Fischer & Glaser 2012). In their study, application of N fertilizer together with biochar had a positive effect on the incorporation of biochar into the larger aggregates, which confirmed our results. In case of B20N80 treatment, the values of WSA_{ma} in the size fractions 3–2 mm (75%) and 5–3 mm (149%) were higher, while the size fraction of 0.5–0.25 mm (27%) was lower than in B20N0. We observed considerably lower content of WSA_{ma} 5–2 mm in B10N80. Dose of 40 kg N ha^{-1} together with 10 and 20 t ha^{-1} of biochar did not have a noticeable effect on WSA_{ma} (except size fraction 3–2 mm in B10N40). Adding nitrogen to the soil can improve microbial activity (Lehmann et al. 2011), increase the intensity of the biochar mineralization processes and increase CEC and active surface area (Yeboah et al. 2009), which results in higher aggregation (Bronic & Lal 2005).

Numerous reports show positive effects of biochar on aggregate stability (Herath et al. 2013; Sun & Lu 2014) and our study fully confirm these findings (Table 2). Biochar in dose of 20 t ha^{-1} increased *St* (55%) compared to B0N0. Addition of manure (Whalen and Chang, 2002), as well as fertilizer application generally improve soil aggregation (Haynes and Naidu, 1998). However, under some conditions fertilizers may also decrease *SOC* concentration, reduce aggregation, and reduce microbial communities compared to manured soils. Yet, using chemical fertilizers often improves soil structure in comparison to unfertilized soils (Munkholm et al., 2002). *St* increased by 24% in B10N80 compared to B10N0. Soil crust is a major structural feature of the surface soil and one of the most important physical properties. Organic fertilizers increase the stock of *SOM*, through which they positively influence the soil physicochemical properties, such as the formation of favourable structure (Czachor et al. 2015). In our study biochar also affected the crusting index (Table 2). Higher amounts of biochar significantly decreased *Ic* values compared to B0N0. The increased mean weight diameter of soil aggregates due to biochar application could be attributed to the increase in the amount of oxidized functional groups after mineralization of biochar (Jien & Wang 2013), which allowed flocculation of the soil particles and biochar, which also meant better structural state.

Relationships between SOM and the soil structure parameters

Pieri (1991) proposed the concept of the critical level of soil organic matter concentration (*St*) for structural stability of the soils. *St* values ranged between 3.28 and 5.08 and biochar and biochar with N had significant influence on *St* (Table 2). Similar to the Pieri (1991) evaluation, our results show lower values of *St*, meaning loss of soil structure and high susceptibility to erosion. The values of *St* increased only after higher amounts of biochar application, confirming the strong relationship between *SOM* and *St* (Table 3). Šimanský & Bajčan (2014) and Rabbi et al. (2015) also present a very strong positive relationship between aggregate stability and organic carbon content. It is a result of the strong linkage between colloidal fractions of soils and *SOM* (Kirkby & Morgan 1980). A very important parameter for the evaluation of soil structure is index of crusting (*Ic*). This is influenced by soil texture, *SOM* concentration and soil management practices (Lal & Shukla 2004). Higher *SOM* content resulted in lower *Ic* values (Table 3). Higher content of labile carbon resulted in lower content of higher size fractions of aggregates (>3 mm). On the other hand, higher C_L content had a positive effect on the increase of WSA_{ma} 2–1 mm.

Conclusion

The results of our study indicate a positive response of *SOM* and soil structure parameters of Haplic Luvisol to biochar application. The most favourable effects on *SOC*, non-labile carbon and soil structure stability were observed when 20 t ha^{-1} of biochar were applied. The same positive effects were observed after the application of 10 t ha^{-1} of biochar in combination with nitrogen at rates of 40 and 80 kg ha^{-1}. Biochar improved aggregation and stability of soil structure, especially labile carbon and carbon lability had positive effects in the size fractions 2–1 mm and 5–2 mm of water-stable macro-aggregates, respectively.

The results of our study indicate that the application of biochar increases SOC content, in both its labile and non-labile forms, and also improves soil structure parameters. This information is very important for farmers, soil management practices can be optimized to avoid environmental degradation of their soils. Based on our findings, we recommend biochar application to pursue sustainable soil management with respect to carbon sequestration and soil structure preservation.

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Table 1. Basic chemical and physical properties of the biochar (biochar certificate Nr.1013069001 provided by company Sonnenerde, Austria).

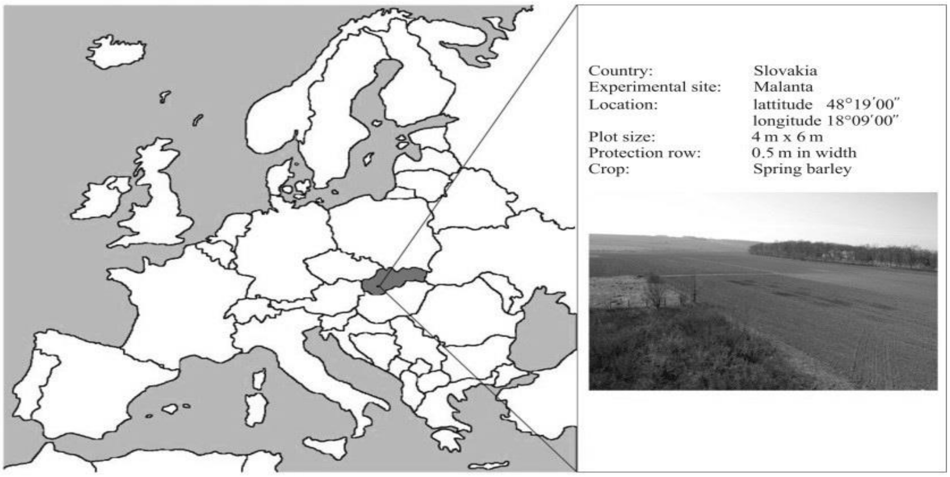
biochar		
pH	(KCl)	8.8
Ca	g kg^{-1}	57
Mg	g kg^{-1}	3.9
K	g kg^{-1}	15
Na	g kg^{-1}	0.7
Total C	g kg^{-1}	53.1
Total N	g kg^{-1}	14
C/N		3.79
SSA	m^{2}g^{-1}	21.7
Ash	%	38.3

Table 2. Statistical evaluation of soil organic matter and parameters of soil structure

	SOC	C_L	C_{NL}	L_C	St	Ic	WSA_{mi}
	g kg^{-1}						%
treatments							
B0N0	12.2 ± 0.85^{a}	1.54 ± 0.31^{a}	10.6 ± 0.74^{a}	0.145 ± 0.03^{c}	3.28 ± 0.23^{a}	2.88 ± 0.03^{d}	30.2 ± 5.41^{b}
B10N0	14.5 ± 1.34^{ab}	1.77 ± 0.24^{a}	12.7 ± 1.44^{ab}	0.142 ± 0.03^{bc}	3.90 ± 0.36^{ab}	2.82 ± 0.04^{cd}	20.5 ± 9.77^{ab}
B20N0	18.9 ± 3.30^{c}	1.89 ± 0.40^{a}	16.9 ± 2.99^{c}	0.112 ± 0.02^{ab}	5.08 ± 0.89^{d}	2.71 ± 0.08^{a}	26.7 ± 11.3^{ab}
B10N40	15.9 ± 1.96^{bc}	1.65 ± 0.41^{a}	14.3 ± 1.88^{bc}	0.116 ± 0.03^{abc}	4.30 ± 0.53^{bc}	2.78 ± 0.05^{bc}	30.4 ± 8.33^{b}
B20N40	17.9 ± 2.90^{cd}	1.89 ± 0.36^{a}	16.0 ± 2.70^{c}	0.119 ± 0.02^{abc}	4.83 ± 0.78^{cd}	2.73 ± 0.07^{ab}	23.8 ± 11.6^{ab}
B10N80	18.0 ± 2.03^{cd}	1.67 ± 0.26^{a}	16.3 ± 2.10^{c}	0.104 ± 0.02^{a}	4.85 ± 0.55^{cd}	2.73 ± 0.05^{ab}	27.4 ± 8.49^{ab}
B20N80	16.4 ± 2.16^{bc}	1.78 ± 0.31^{a}	14.7 ± 1.87^{bc}	0.121 ± 0.01^{abc}	4.43 ± 0.58^{bcd}	2.70 ± 0.06^{abc}	17.3 ± 5.23^{a}

Table 3. Correlation coefficients between soil organic matter and soil structure parameters

	St	Ic	>5	5–3	3–2	2–1	1–0.5	0.5–0.25	WSA_{mi}
SOC	1.000^{***}	-0.999^{***}	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
C_L	0.425^{**}	-0.417^{**}	-0.442^{**}	n.s.	n.s.	0.382^{*}	n.s.	n.s.	n.s.
L_C	-0.673^{***}	0.683^{***}	-0.502^{***}	-0.355^{*}	n.s.	n.s.	n.s.	n.s.	n.s.



Country: Slovakia
Experimental site: Malanta
Location: lattitude 48°19′00″
longitude 18°09′00″
Plot size: 4 m x 6 m
Protection row: 0.5 m in width
Crop: Spring barley

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

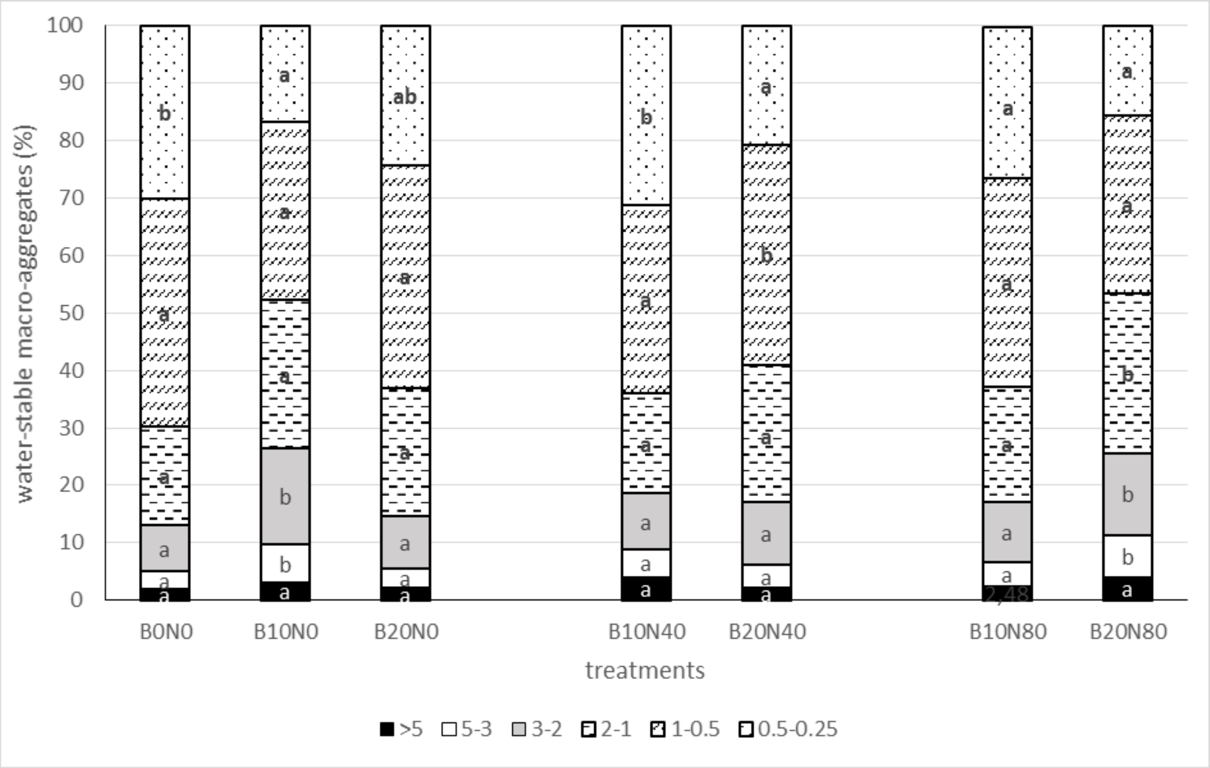


Fig. 2. Content of individual size-fraction of water-stable macro-aggregates.

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