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The influence of soil organic matter fractions on aggregates stabilization in agricultural and forest soils of selected Slovak and Czech hilly lands

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Abstract
Purpose Because the stability of soil aggregates is affected by many factors, we studied aggregates formed in forest and agricultural soils in different soil types (Cambisols, Luvisols, Chernozems). We evaluated: (1) the differences in water-stable aggregates (WSA) as related to soil type and land management and (2) the relationships between quantitative and qualitative parameters of soil organic matter (SOM), particle-size distribution and individual size classes of WSA.
Materials and methods Soil samples were taken from three localities (Soběšice, Báb, Vieska nad Žitavou). Each study locality included both a forest and an agricultural soil-sampling area.
Results and discussion We found that in forest soils, the proportion of water-stable macroaggregates (WSA_{ma}) relative to water-stable microaggregates (WSA_{mi}) was greater than in agricultural soils. When all soils were assessed together, positive statistically significant correlations were observed between the size classes $WSA_{ma} > 1$ mm and organic carbon (C_{org}) content; however, the WSA_{mi} content was negatively correlated with C_{org} content. Favorable humus quality positively influenced the stabilization of $WSA_{ma} > 5$ mm; however, we found it had a negative statistically significant effect on stabilization of WSA_{ma} 1–0.25 mm. In agricultural soils, the stabilization of WSA_{ma} was associated with humified, i.e., stable SOM. The WSA_{ma} content was highly positively influenced mainly by fulvic acids bound with clay and sesquioxides; therefore, we consider this humus fraction to be a key to macroaggregate stability in the studied agricultural soils. On the other side, all fractions of humic and fulvic acids participated on the formation of WSA_{ma} in forest soil, which is a major difference in organic stabilization agents of macroaggregates between studied forest and agricultural soils. Another considerable difference is that WSA_{mi} in agricultural soils were stabilized primarily with humic acids and in forest soils by fulvic acids. Moreover, in forest soils, a higher content of labile carbon in WSA had a positive effect on formation of WSA_{mi}.
Conclusions The observed changes in individual size classes of WSA and interactions between SOM, particle-size distribution, and WSA have a negative impact on soil fertility and thereby endanger agricultural sustainability.

Keywords Agricultural soils · Forest soils · Soil organic matter fractions · Soil structure

1 Introduction

Soil structure is important for many soil environmental processes and is the key factor in soil quality (Roger-Estrade et al. 2010; Garbout et al. 2013). Indeed, stable soil structure is essential for supporting life and moderating environmental quality (Bronick and Lal 2005). In particular, soil structure is one of the most important factors in the stabilization of soil organic carbon, because this one is a significant binding agent that associates mineral particles together into aggregates (Chaplot and Cooper 2015; Rabbi et al. 2015). Soil aggregates are the basic units of soil structure (Lal and Shukla 2004).

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Among other things, soil aggregation, i.e., formation of soil aggregates, protects soil from erosion and enhances plant growth via increased aeration and better retention of water and nutrients (Lal and Shukla 2004). In addition, aggregates can shelter carbon inside of them (Šimanský and Bajčan 2014), which can be a key in using carbon sequestration to mitigate global climate change. Thus, the assessment of a given soil's aggregation is crucial in evaluating its potential for forming the optimal soil structure for plant growth in forest and agricultural soils as well as its capability to perform carbon sequestration.

The stability of soil aggregates is one of the most important ways in which they can be characterized (Six et al. 2004). Stabilization is influenced by different factors. These include soil intrinsic factors such as electrolyte concentration, types of exchangeable cations (Paradelo et al. 2013), clay mineralogy (Bronic and Lal 2005), carbonate contents (Vaezi et al. 2008), soil organic matter (Saha et al. 2011; Šimanský and Bajčan 2014), and Fe and Al oxides (Barthes et al. 2008). All of these are influenced by climate, soil formation processes (wet-dry and freeze-thaw cycles), biological factors, and soil management practices (Balashov and Buchkina 2011; Kurakov and Kharin 2012). Formation and stability of natural soil aggregates are thus overall affected by dozens of factors, and their individual effects are difficult to distinguish (Józefaciuk and Czachor 2014).

Given the numerous factors that influence aggregate stability, it is not surprising that this stability depends upon soil type, even for individual soil horizons (Šimanský and Bajčan 2014; Bryk 2016) and land use. Formation and subsequent stabilization of soil aggregates is specifically bound to the soil type. For example, the hierarchical formation of aggregates in Andisol (Asano and Wagai 2014) is different than in Oxisol (Field et al. 2006). Many studies suggest that intensive cultivation disrupts the soil structure, mainly during unfavorable moisture state of soil (Šimanský 2012; Schweizer et al. 2017). Soil cultivation affects soil aggregation directly by physical disruption of the macroaggregates and indirectly through alteration of biological and chemical properties (Barto et al. 2010). As is above-mentioned, soil management plays an important role in soil structure formation in arable soils (Balashov and Buchkina 2011). It is already well known that soil management practices influence the content and quality of soil organic matter (Šimon et al. 2009), which is a very important factor of water-stable aggregates (WSA) formation (Onweremadu et al. 2007; Krol et al. 2013). Macroaggregates are also formed and stabilized by biological factors, such as roots and fungal hyphae and by products of microbial synthesis and decay (Lado et al. 2004); therefore, more WSA are often determined in grassland than in arable lands (Šimanský 2012), or in forest soils compared to arable soils (Polláková 2012). For example, the ideal soil structure is formed by predominantly crumb-like aggregates, best formed when organic matter is naturally high (> 2% organic carbon content) and well humified (Greenland et al. 1975). Soil organic matter (SOM) and its labile fractions as well as soil clay minerals, bacteria, and fungi are important agents for the formation and stabilization of WSA (Tisdall and Oades 1982; Six et al. 2004). SOM affects the water stability of aggregates by decreasing their wettability and increasing their mechanical strength (Onweremadu et al. 2007).

Stable, waterproof soil structure is essential for good soil quality. However, intensive cultivation causes a decrease of SOM content and considerably disrupts mainly soil macroaggregates. Therefore, we assume that the factors stabilizing soil aggregates in agricultural soil will differ from that of forest soil. In the present study, we tried to identify and compare which components of SOM stabilize the individual size classes of WSA in different soil types as well as in forest and agricultural soils. In particular, we evaluated: (i) the differences in WSA as related to soil types and land use and (ii) the relationships between SOM, particle-size distribution, and individual size classes of WSA.

2 Materials and methods

2.1 Study localities and their brief descriptions

Our studies were carried out on soil samples obtained from three localities. Each study locality included both a forest and an agricultural soil-sampling area.

The first locality, Soběšice (49°14′52.3″N 16°36′33.9″E), is situated near Brno (Czech Republic). The geological substrates of this area are granodiorites. The mean annual temperature there is 8 °C and the mean annual precipitation is 530 mm. For Soběšice, the forest soil came from a mixed, 101-year-old forest stand—consisting of 69% of oak (*Quercus petraea*/Mattuschka/Liebl.), 22% of hornbeam (*Carpinus betulus* L.), and 9% of pine (*Pinus sylvestris* L.), with a litter thickness of 5 cm and the total area of forest with these conditions occupying 14 ha. The Soběšice agricultural soil was sampled from an extensive meadow dominated by the grasses *Poa pratensis* L. and *Dactylis glomerata* L. with a total area of 3 ha. The land was used as arable for intensive plant production until the year 2010. Morphological characters of tillage homogenization of topsoil are still well recognized.

The second locality, Báb (48°18′5.50″N, 17°53′56.59″E) is near the town of Nitra, in the western part of Slovakia. The soils at Báb developed on calcareous loess parent material. The average annual temperature there is 10.2 °C and the mean annual precipitation is 539 mm (Szombathová and Zaujec 2001). For Báb, the forest soil was sampled from deciduous forest, at least 200 years old, with a predominance of oaks and hornbeams (*Quercus robur*, L., *Carpinus betulus*, L.), with a

5-cm thick litter layer and a total forest area of 7 ha (MAB research area). The Báb site from which agricultural soil was sampled consisted of arable soil with an area of 25 ha that has been plowed yearly for minimally 100 years. During sampling, maize was being grown on the field, with the forecrop having been winter wheat.

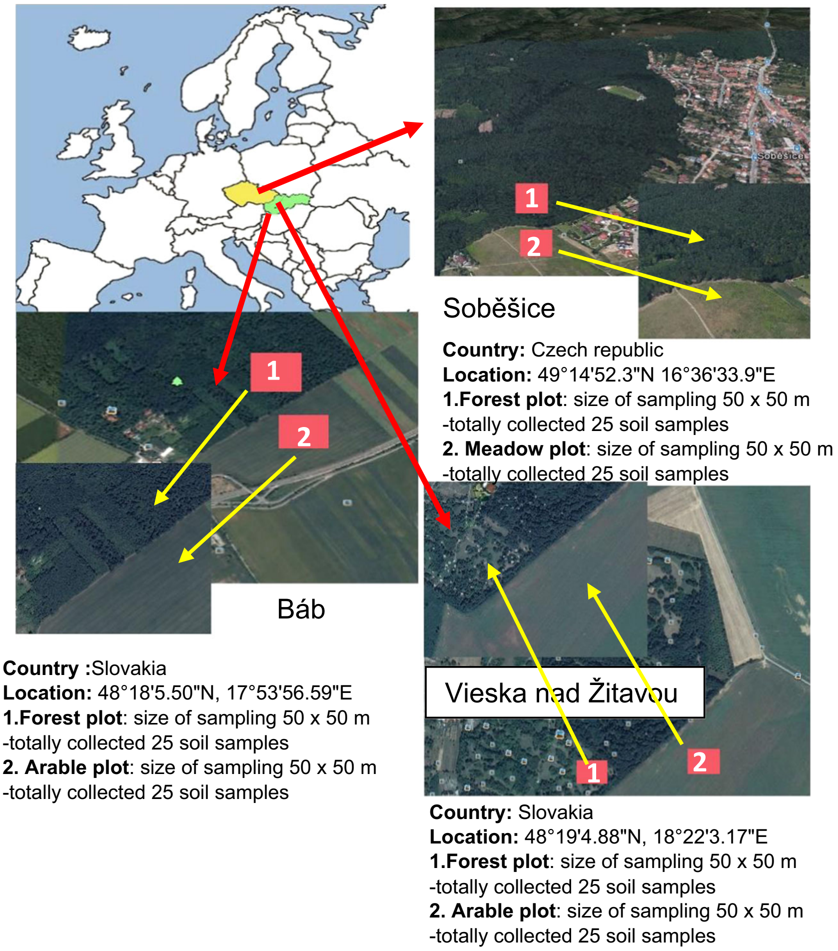
The last locality, Vieska nad Žitavou (48°19′4.88″N, 18°22′3.17″E) is also in the western part of Slovakia, near the town of Zlaté Moravce. The soils in Vieska nad Žitavou developed on a late Tertiary geological formation, represented by Neogene clays, sands, and rubble sands which are almost all covered by wind-deposited loess. The mean annual temperature in this area is 10.6 °C and the mean annual precipitation is 541 mm (Polláková 2013). At Vieska nad Žitavou, the forest soil was collected from an area featuring thujas (*Thuja occidentalis* "Malonyana," L.) at least 70 years old, with a 12-cm thick litter layer. The total area of the forest is 0.30 ha (research area—part of arboretum). The field from which agricultural soil was sampled had arable soil, with an area of 85 ha, which has been plowed yearly for at least 100 years. Maize was being grown on the field at the time of sampling; the forecrop had been sunflower.

2.2 Soil sampling and analyses

At each study locality, we established two 2500-m^2 soil-sampling plots sites (50 × 50 m), one in the forest area and one in the agricultural area, thus yielding a total of six such plots (Fig. 1). Because soil-climatic conditions were almost the same within each pair of plots, we could make comparisons across the two land use types (forest, agricultural).

Soil profiles for each sampling plot were described, with soils classified according to the World Reference Base for Soil Resources (WRB 2006) based on whole-profile soil morphology. General descriptions of the studied soils are presented in Table 1.

Soil samples were taken from all sites in the networks 10 × 10 m from layer 0–30 cm (in forest soils, the litter layer was removed). We collected 25 soil samples from each plot, yielding totals of 50 soil samples from each locality (25 soil samples from forest plot + 25 soil samples from arable plot) and 150 samples overall (3 localities × 50 soil samples from one locality). After removing plant material (before mechanical and chemical analyses), the soil samples were dried at laboratory temperature (20 °C) and ground up.



Country: Czech republic
Location: 49°14'52.3"N 16°36'33.9"E
1.Forest plot: size of sampling 50 x 50 m
-totally collected 25 soil samples
2. Meadow plot: size of sampling 50 x 50 m
-totally collected 25 soil samples

Country :Slovakia
Location: 48°18'5.50"N, 17°53'56.59"E
1.Forest plot: size of sampling 50 x 50 m
-totally collected 25 soil samples
2. Arable plot: size of sampling 50 x 50 m
-totally collected 25 soil samples

Country: Slovakia
Location: 48°19'4.88"N, 18°22'3.17"E
1.Forest plot: size of sampling 50 x 50 m
-totally collected 25 soil samples
2. Arable plot: size of sampling 50 x 50 m
-totally collected 25 soil samples

Fig. 1 Site location and sampling areas

Table 1 Description of investigated soils

Locality	Soil type	Land use	Soil characteristics						
			Sand (%)	Silt	Clay	C_{org}	BS	pH_{KCl}	C_{HA}/C_{FA}
Soběšice	Cambisol	Forest	22.0 ± 2.3	51.5 ± 6.4	26.5 ± 3.9	4.15 ± 0.7	76.6 ± 5.4	5.23 ± 1.2	0.85 ± 0.18
		Grassland	39.6 ± 4.5	42.5 ± 3.7	17.9 ± 2.4	2.21 ± 0.3	86.5 ± 3.5	5.81 ± 0.8	0.63 ± 0.07
Vieska nad Žitavou	Luvisol	Forest	14.3 ± 2.1	57.2 ± 7.8	28.5 ± 2.8	4.66 ± 0.5	98.7 ± 6.9	7.25 ± 1.4	0.93 ± 0.22
		Arable	13.9 ± 1.8	59.6 ± 6.5	26.5 ± 4.6	1.27 ± 0.2	78.7 ± 2.9	5.31 ± 0.3	0.60 ± 0.06
Báb	Chernozem	Forest	14.6 ± 1.1	59.6 ± 4.3	25.8 ± 5.8	4.51 ± 0.6	97.1 ± 3.9	6.55 ± 0.9	1.18 ± 0.18
		Arable	14.6 ± 0.9	59.2 ± 2.9	26.2 ± 3.7	1.93 ± 0.2	91.4 ± 2.7	6.12 ± 0.2	1.22 ± 0.09

Means and standard deviations, *Corg* soil organic carbon, pH_{KCl} soil pH in 1 mol dm^{-3} of KCl (1:2.5—soil:KCl), *BS* base saturation, $C_{HA}:C_{FA}$ the carbon of humic acids to carbon of fulvic acids ratio

For the present study, each soil sample was divided into six particle-size classes by the pipette method as is described in Fiala et al. (1999). Briefly, the particle-size distribution was determined after dissolution of $CaCO_3$ with 2 mol dm^{-3} of HCl and decomposition of the organic matter with 6% H_2O_2. After repeated washing, the samples were dispersed using a solution of 0.06 mol dm^{-3} of $(NaPO_3)_6$ and 0.075 mol dm^{-3} of Na_2CO_3.The classes obtained were designated as coarse sand (2–0.25 mm), medium and fine sand (0.25–0.05 mm), coarse silt (0.05–0.01 mm), medium and fine silt (0.01–0.001 mm), and clay (< 0.001 mm). The content of particles < 0.01 mm was calculated as the sum of medium and fine silt and clay content. Content of sand was represented sum of coarse sand with medium and fine sand. Content of silt was presented by sum of coarse silt with medium and fine silt.

The content of soil organic carbon (C_{org}) was determined using the wet combustion method of Tyurin (Dziadowiec and Gonet 1999), oxidizing organic matter using a mixture of 0.07 mol dm^{-3} of H_2SO_4 and $K_2Cr_2O_7$ with titration using 0.01 mol dm^{-3} of Mohr's salt. Fractional composition of humus was analyzed using the Tyurin method as modified by Ponomareva and Plotnikova (Dziadowiec and Gonet 1999). The optical characteristics of humus quality were assessed as absorbance of humic substances and humic acids (0.1 mol dm^{-3} of NaOH) measured at wavelength of 465 and 650 nm using a Jenway Model 6400 spectrophotometer. Since humic substances (C_{HS}) and humic acids (C_{HA}) contain great number of chromophores and do not create separate peaks in UV VIS range, in determining the chemical structure of C_{HS} and C_{HA}, very useful are the analysis of the so called color quotients of humic substances ($Q^{4/6}{}_{HS}$) and humic acids ($Q^{4/6}{}_{HA}$), calculated as ratios of absorbances 465 nm (the content of materials at the beginning stage of transformation) and 650 nm (strongly humified organic materials), which decreases with increasing molecular weight and condensation (Stevenson 1982).

To maintain the soil aggregation, soil samples for aggregate determination were carefully taken using a spade. They were mixed to produce an average representative sample from each stand. Every root and large piece of litter was removed. The collected soil samples were transported to the laboratory and large clods were gently broken up along natural fracture lines, followed by air-drying at laboratory temperature (20 °C) to obtain undisturbed soil samples. To determine water-stable aggregates (WSA), we used the AS 200 device (Retsch®). The aggregates were treated with fast wetting by water. Sieving of soil samples was done with four sieves with mesh of 5, 3.15, 1, and 0.25 mm. On the top sieve, the clamping cover with spray nozzle was used for fast wet sieving and after the last sieve, the base pan with water drain were given, and aggregates were then sieved 10 min. After the sieving process, the classes were transferred from the individual sieves to suitable filters (e.g., filter paper) and dried in a drying oven at 45 °C. The material retained was quantified in each sieve except for microaggregates, WSA_{mi} (i.e., water-stable aggregates < 0.25 mm), for which the content was calculated as the difference between total weight of the soil sample and the sums of the macroaggregates, WSA_{ma} (i.e., water-stable aggregates > 0.25 mm). In all size classes of WSA (in WSA_{ma} as well as in WSA_{mi}), we analyzed the total organic carbon by the Tyurin method as mentioned above and the labile carbon by the Loginow method (Loginow et al. 1987). Using this method, the labile carbon content (C_L) was extracted from samples containing 1 g of individual particle-size classes of WSA by shaking in 50 mL solution of 0.005 mol dm^{-3} $KMnO_4$ and 0.0025 mol dm^{-3} H_2SO_4 for 2 h. After centrifugation, the C_L was determined by oxidation of 0.07 mol dm^{-3} H_2SO_4 and $K_2Cr_2O_7$ with titration using 0.05 mol dm^{-3} Mohr's salt. We also analyzed hot-water extractable carbon (C_{HWD}) in WSA (Kőrschens 2002). The C_{HWD} was extracted from samples containing 5 g of individual particle-size classes of WSA by shaking in 50 mL of distilled water with 80 °C for 16 h.

After filtering, the C_{HWD} was determined by oxidation of 0.07 mol dm^{-3} H_2SO_4 and $K_2Cr_2O_7$ with titration using 0.01 mol dm^{-3} Mohr's salt.

2.3 Statistical analyses

Statistical analysis was performed using the Statgraphics Centurion XV.I program (Statpoint Technologies, Inc., USA). The different aggregate classes were analyzed with a multiple analysis of variance (ANOVA) with soil type and land use as two factors. A least significant difference test (LSD) was used for comparisons between means at $P \leq 0.05$. Relationships between SOM, particle-size distribution, and individual size classes of WSA were then determined.

3 Results and discussion

3.1 Contents of water-stable aggregates as related to soil type and land use

When soils from all studied stands were assessed together, soil type had a statistically significant influence only on the contents of size classes of water-stable macroaggregates (WSA_{ma}) > 3.15 mm and water-stable microaggregates (WSA_{mi}), while contents of WSA_{ma} 3.15–0.25 mm were very similar (Table 2). The content of WSA_{ma} > 5 mm in the Cambisol was almost four times higher than in the Chernozem and two times higher than in the Luvisol. On the other hand, the Cambisol contained 2.2 and 2.4 times less WSA_{mi} than the Chernozem and Luvisol, respectively. The content of WSA depends on the soil type (Field et al. 2006; Asano and Wagai 2014; Šimanský and Bajčan 2014), soil management practices (Gaida et al. 2013; Slowinska-Jurkiewicz et al. 2013), and land-use type (Balashov and Buchkina 2011; Polláková 2012; Liu et al. 2014). Forest soils (Khurakov and Kharin 2012; Polláková 2012) and soils after afforestation (Vopravil et al. 2014) show higher contents of macroaggregates relative to microaggregates than arable soils do. The same results were observed in the present study when soils from all studied stands were assessed together. In agricultural soils, the content of WSA_{mi} increased by 123% as compared to forest soils. In contrast, in forest soils, the representations of WSA_{ma} size classes, > 5, 5–3.15, 3.15–1, and 1–0.25 mm, were higher than in agricultural soils by 169%, 117%, 96%, and by 24%, respectively.

3.2 Relationships between soil organic matter characteristics, textural properties, and individual size classes of water-stable aggregates in all investigated soil types and land uses

Table 3 summarizes correlations between the individual WSA size classes and soil organic matter (C_{org}; C_L; C_{HWD}; C_{HA}; and C_{FA} in WSA) when all studied soil types and land uses were assessed together. In general, soil organic matter (SOM) is the most important internal factor affecting the binding of soil mineral particles together (Blanco-Canqui and Lal 2007; Krol et al. 2013; Šimanský and Bajčan 2014; Rabbi et al. 2015). Positively, statistically significant correlations were observed between the size classes of WSA_{ma} > 1 mm and contents of organic carbon (C_{org}); however, the content of WSA_{mi} was negatively correlated with C_{org}. Consistent with this founding, some studies have reported carbon content to be higher in the macroaggregates than in microaggregates (Carter 1992; Spaccini et al. 2001; Liu et al. 2014). Tisdall and Oades (1982) found that in soil with a total organic carbon content of > 2%, the most important stabilizing agent of aggregates in size classes 20–250 and > 2000 μm are roots and hyphae. As shown by Blavet et al. (2009), macroaggregate stability is positively correlated with soil C_{org} content as a result of strong linkage between the colloidal fractions of soils.

Table 2 Content of individual size fractions of water-stable aggregates in investigated soil types

Size fractions of water-stable aggregates in mm					
	> 5	5–3.15	3.15–1	1–0.25	< 0.25
Soil type					
Cambisols	38.08 ± 9.45^{b}	15.31 ± 4.88^{b}	13.09 ± 2.49^{a}	7.79 ± 2.22^{a}	25.75 ± 8.25^{a}
Luvisols	13.96 ± 5.87^{a}	$11.44 \pm 5.44^{a,\ b}$	11.00 ± 4.68^{a}	7.13 ± 3.49^{a}	56.48 ± 12.1^{b}
Chernozems	8.05 ± 3.79^{a}	8.80 ± 2.44^{a}	12.58 ± 4.07^{a}	8.84 ± 2.96^{a}	61.75 ± 9.22^{b}
ANOVA	0.0000	0.0021	0.5454	0.6445	0.0000
Land use					
Forest soils	29.19 ± 8.52^{b}	16.22 ± 4.22^{b}	16.19 ± 5.23^{b}	8.77 ± 2.86^{a}	29.63 ± 4.89^{a}
Agricultural soils	10.86 ± 2.75^{a}	7.48 ± 1.19^{a}	8.25 ± 2.55^{a}	7.06 ± 2.92^{a}	66.35 ± 11.9^{b}
ANOVA	0.0000	0.0000	0.0000	0.2620	0.0000

Different superscript letters between lines (a, b) indicate significant differences at $P \leq 0.05$ according to LSD multiple-range test

Table 3 Correlation coefficients (*r*) between average values of soil organic matter parameters and individual size fractions of water-stable aggregates and between average values of particle-size distribution and individual size fractions of water-stable aggregates in all soil types and land uses together

		WSA_{mi}	WSA_{ma}			
		Size fractions of water-stable aggregates in mm				
		< 0.25	> 5	5–3.15	3.15–1	1–0.25
C_{org}		-0.746^{***}	0.519^{**}	0.795^{***}	0.746^{***}	n.s.
C_{HA}:C_{FA}		n.s.	0.715^{***}	n.s.	n.s.	-0.620^{***}
$Q^{4/6}{}_{HS}$		-0.522^{**}	0.535^{**}	0.551^{**}	n.s.	n.s.
$Q^{4/6}{}_{HA}$		-0.524^{**}	0.591^{**}	0.482^{**}	n.s.	n.s.
C_{HA1}		n.s.	n.s.	-0.367^{*}	-0.628^{***}	-0.469^{**}
C_{HA2}		0.879^{***}	-0.763^{***}	-0.827^{***}	-0.569^{**}	n.s.
C_{HA3}		0.677^{***}	n.s.	-0.735^{***}	-0.870^{***}	-0.522^{**}
ΣC_{HA}		0.713^{***}	-0.361^{*}	-0.754^{***}	-0.870^{***}	-0.522^{**}
C_{FA1a}		0.496^{**}	-0.417^{*}	n.s.	-0.421^{*}	n.s.
C_{FA1}		n.s.	n.s.	n.s.	n.s.	n.s.
C_{FA2}		0.519^{**}	-0.724^{***}	-0.378^{*}	n.s.	n.s.
C_{FA3}		0.567^{**}	-0.702^{***}	n.s.	n.s.	n.s.
ΣC_{FA}		0.645^{***}	-0.754^{***}	-0.433^{*}	n.s.	n.s.
C_{org} in WSA		-0.577^{***}	n.s.	0.658^{***}	0.686^{***}	n.s.
C_{L} in WSA		-0.495^{**}	n.s.	0.617^{***}	0.691^{***}	n.s.
C_{HWD} in WSA		-0.662^{***}	0.626^{***}	0.624^{***}	0.444^{*}	n.s.
Particle-size distribution in mm	> 0.25	n.s.	n.s.	n.s.	n.s.	n.s.
	0.25–0.05	n.s.	-0.514^{*}	n.s.	n.s.	n.s.
	0.05–0.01	n.s.	0.453^{*}	-0.553^{*}	n.s.	n.s.
	0.01–0.001	n.s.	n.s.	n.s.	n.s.	n.s.
	< 0.001	n.s.	n.s.	n.s.	n.s.	n.s.
	< 0.01	n.s.	n.s.	n.s.	n.s.	n.s.

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $n = 30$

n.s. non-significant, C_{org} soil organic carbon, C_{HA}:C_{FA} the carbon of humic acids to carbon of fulvic acids ratio, $Q^{4/6}{}_{HS}$ color quotient of humic substances, $Q^{4/6}{}_{HA}$ color quotient of humic acids, C_{HA1} humic acids bound with monovalent cations, C_{HA2} humic acids bound with bivalent cations, C_{HA3} humic acids bound with the clay and sesquioxides, ΣC_{HA} total content of humic acids, C_{FA1a} free fulvic acids, C_{FA1} fulvic acids bound with monovalent cations, C_{FA2} fulvic acids bound with bivalent cations, C_{FA3} fulvic acids bound with the clay and sesquioxides, ΣC_{FA} total content of fulvic acids, C_{org} *in WSA* soil organic carbon in water-stable aggregates, C_{L} *in WSA* labile carbon in water-stable aggregates, C_{HWD} *in WSA* hot water-soluble carbon in water-stable aggregates, WSA_{mi} water-stable microaggregates, WSA_{ma} water-stable macroaggregates

The higher total content of humic acids (C_{HA}) and fulvic acids (C_{FA}) was negatively correlated with WSA_{ma}, but positively with WSA_{mi} (Table 3) which suggests that WSA_{mi} were predominantly stabilized by more stable fractions of soil organic matter transformed by humification process, i.e., humic acids and fulvic acids, while stabilization of WSA_{ma} was mostly by labile SOM fractions—labile carbon (C_L) and hot-water soluble carbon (C_{HWD}) when all soil types (Chernozems, Luvisols, Cambisols) and land uses (forest, agricultural) were assessed together. The finding of positive correlation of more stable, humified organic matter with microaggregates is in agreement with many studies such as reported by Tisdall and Oades (1982), Yu et al. (2012), Liu et al. (2014), and Cao et al. (2016). On the other side, less studies were published about the negative correlation of C_{HA} and C_{FA} with WSA_{ma} (Shujie et al. 2009), however, positive correlation between C_{HA} and C_{FA} with WSA_{ma} (Kimura et al. 2017; Samahadthai et al. 2010; Li et al. 2017), while positive correlation between labile fractions of organic matter and WSA_{ma} is generally recognized (Oades 1993; Shujie et al. 2009; Liu et al. 2014; Li et al. 2017).

From previous studies, it can be recognized that many scientists are concerned with the role of humus in soil aggregation. But, we did not find available literature concerning individual humic and fulvic acid fractions in soil aggregation and therefore, we assume this work as innovative. Results in Table 3 showed that similarly, as for total content of C_{HA} and C_{FA}, statistically significant trends of increased WSA_{mi} content were found also in individual humic acid fractions, mainly in (C_{HA3}) humic acids bound with the clay and sesquioxides and in those (C_{HA2}) bound with bivalent cations. A similar trend was also found in humic acids bound with monovalent cations (C_{HA1}). Compared to C_{HA}, less significance was found in fulvic acids between the size of WSA and C_{FA} fractions. However, the trends were similar, meaning that increased WSA_{mi} content was positively correlated with C_{FA1a} (free fulvic acids), C_{FA2} (bound with bivalent cations), and C_{FA3} (bound with clay and sesquioxides). In contrast, both the content of C_{FA} overall and its individual C_{FA} fractions showed negative correlations with the amount of WSA_{ma} with a size of > 0.25 mm.

The C_{HA}:C_{FA} ratio has been used as an index of the degree of humification (Stevenson 1982) in individual aggregate size classes. The results in Table 3 follows that increased degree of humification was highly significantly correlated with WSA_{ma} > 5 mm but the significance was lower for the others size classes of WSA_{ma}, such that correlation was a negative significant for size class 1–0.25 mm, but non-significant for other micro- and macroaggregates sizes. When all soils were assessed together, the higher humus condensation (low color quotients $Q^{4/6}{}_{HS}$ and $Q^{4/6}{}_{HA}$) positively affected the stabilization of WSA_{mi} and negative WSA_{ma} > 3.15 mm. According to Stevenson (1982), low $Q_{4/6}$ ratio may be an indicative of more humified and highly condensed (aromatic) substances and more antient origin. Similar findings as were reached in this study were found also by Šimanský et al. (2013) for Rendzic Leptosol. Undoubtedly, as was reported by Rabbi et al. (2010), microaggregates have the capacity to significantly slow SOM decomposition over physical protection (Peth et al. 2008) and physico-chemical protection (Kögel-Knabner et al. 2008) which is reflected in their higher stability (Šimanský et al. 2013). Results in Table 3 showed that in studied soils, the WSA_{mi} were stabilized by deeply transformed, i.e., humified and highly condensed organic matter (positive correlation with individual fractions of C_{HA} and C_{FA}, negative correlation with $Q^{4/6}{}_{HS}$, $Q^{4/6}{}_{HA}$, C_L, and C_{HWD}), while for WSA_{ma} was the trend opposite, and the stability was supported mainly by labile fractions of SOM (C_L and C_{HWD}). Many studies (Tisdall and Oades 1982; Yu et al. 2012; Liu et al. 2014) confirmed WSA_{mi} stabilization mainly by stable fractions of SOM, while WSA_{ma} by more labile fractions of SOM. On the other side, Cao et al. (2016) and Li et al. (2017) found positive correlation between C_{HA}, C_{FA}, humines, not only with WSA_{mi} but also with WSA_{ma}.

Aggregation is further enhanced by such biological and chemical processes as flocculation and cementation by organomineralic bonding (Dexter 1988). Soil texture is another important factor in aggregation (Lal and Shukla 2004; Paradelo et al. 2013). Assessing all soils together, particle-size distribution was not the key factor affecting aggregates stability (Table 3); therefore, we have not been studying the relationships between particle-size distribution and aggregates separately in forest and in agricultural soils.

3.3 Relationships between soil organic matter characteristics and individual size classes of water-stable aggregates in forest soils

When the studied soils were assessed separately by land use type, some differences were observed. In forest soils, positive correlations between C_{org} and both WSA_{mi} and WSA_{ma} 5–3.15 mm were found and a negative correlation between C_{org} and WSA_{ma} > 5 mm (Table 4). In forest soils, for aggregation of macroaggregates, responsible are mainly roots and hyphae (Tisdall and Oades 1982) and obviously, labile forms of SOM (Carter 1992; Liu et al. 2014; Li et al. 2017). Contrary to this knowledge, in our study, the stabilization of WSA_{ma} by labile SOM was not significant and moreover, negative correlation between labile carbon (C_L) and WSA_{ma} > 5 mm and positive correlation between C_L and WSA_{mi} were found.

Higher humus condensation and stability (C_{HA}:C_{FA} ratio) had a highly significantly positive effect on the content of WSA_{ma} > 5 mm but a negative influence on the content of WSA_{ma} < 3.15 mm and WSA_{mi}. Negative correlation between WSA_{mi} and C_{HA}:C_{FA} suggests that microaggregate stability was highly significantly influenced by fulvic acids, mainly by C_{FA3}, C_{FA2}, and C_{FA1a} fractions.

Based on the negative correlation of WSA_{ma} with optical parameters of humic substances ($Q^{4/6}{}_{HS}$) and humic acids ($Q^{4/6}{}_{HA}$), we can conclude that more condensed (humified) fractions of humus dominated in the formation of WSA_{ma} in size classes 3.15–0.25 mm, while the positive correlation with WSA_{ma} (size > 3.15 mm) suggests that less humified fractions of humus promoted WSA_{ma} in size classes > 3.15 mm formation. These results imply that in the studied forest soil, the humus fractions highly enhanced the stability of WSA_{ma}, mainly C_{HA2}, C_{FA2}, and C_{FA3}, but also C_{HA1}, C_{FA1a}, and C_{FA1}, thus on WSA_{ma} stabilization contributed all fractions of deeply transformed, i.e., humified organic matter. Great importance of humic substances in aggregate stability was confirmed by studies of Samahadthai et al. (2010) and Li et al. (2017). Kimura et al. (2017) noted that macroaggregates positively correlated with humic acids and aromatic compounds, confirming the role of aromatic humic substances in the macroaggregates formation.

3.4 Relationships between soil organic matter characteristics and individual size classes of water-stable aggregates in agricultural soils

Correlation coefficients between average values of SOM parameters and individual size classes of WSA in agricultural soils are summarized in Table 5. Higher C_{org} content resulted in higher content of WSA_{ma} > 3.15 mm and also lower content of WSA_{mi}. In agricultural soils, higher degree of humification (C_{HA}:C_{FA} ratio) ($r = 0.996$, $P \leq 0.001$) and degree of humic substances and humic acids

Table 4 Correlation coefficients (r) between average values of soil organic matter parameters and individual size fractions of water-stable aggregates in forest soils

	WSA_{mi}	WSA_{ma}			
	Size fractions of water-stable aggregates in mm				
	< 0.25	> 5	5–3.15	3.15–1	1–0.25
C_{org}	0.984^{***}	-0.868^{***}	0.782^{***}	n.s.	n.s.
C_{HA}:C_{FA}	-0.914^{***}	0.997^{***}	n.s.	-0.894^{***}	-0.757^{***}
$Q^{4/6}{}_{HS}$	n.s.	n.s.	0.640^{*}	-0.862^{***}	-0.960^{***}
$Q^{4/6}{}_{HA}$	n.s.	0.671^{**}	n.s.	-0.957^{***}	-0.999^{***}
C_{HA1}	n.s.	0.649^{**}	n.s.	-0.948^{***}	-0.997^{***}
C_{HA2}	0.600^{*}	-0.833^{***}	n.s.	0.999^{***}	0.980^{***}
C_{HA3}	n.s.	0.733^{**}	n.s.	-0.979^{***}	-0.999^{***}
ΣC_{HA}	n.s.	0.628^{*}	n.s.	-0.939^{***}	-0.995^{***}
C_{FA1a}	0.542^{*}	n.s.	0.989^{***}	n.s.	-0.528^{*}
C_{FA1}	-0.747^{**}	0.927^{***}	n.s.	-0.988^{***}	-0.920^{***}
C_{FA2}	0.611^{*}	-0.840^{***}	n.s.	0.999^{***}	0.977^{***}
C_{FA3}	0.993^{***}	-0.974^{***}	0.568^{*}	0.720^{**}	0.528^{*}
ΣC_{FA}	0.997^{***}	-0.965^{***}	0.599^{*}	0.693^{**}	n.s.
C_{org} in WSA	n.s.	n.s.	n.s.	n.s.	n.s.
C_L in WSA	0.541^{*}	-0.557^{*}	n.s.	n.s.	n.s.
C_{HWD} in WSA	n.s.	n.s.	n.s.	n.s.	n.s.

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $n = 15$

n.s. non-significant, C_{org} soil organic carbon, C_{HA}:C_{FA} the carbon of humic acids to carbon of fulvic acids ratio, $Q^{4/6}{}_{HS}$ color quotient of humic substances, $Q^{4/6}{}_{HA}$ color quotient of humic acids, C_{HA1} humic acids bound with monovalent cations, C_{HA2} humic acids bound with bivalent cations, C_{HA3} humic acids bound with the clay and sesquioxides, ΣC_{HA} total content of humic acids, C_{FA1a} free fulvic acids, C_{FA1} fulvic acids bound with monovalent cations, C_{FA2} fulvic acids bound with bivalent cations, C_{FA3} fulvic acids bound with the clay and sesquioxides, ΣC_{FA} total content of fulvic acids, C_{org} *in WSA* soil organic carbon in water-stable aggregates, C_L *in WSA* labile carbon in water-stable aggregates, C_{HWD} *in WSA* hot water-soluble carbon in water-stable aggregates, WSA_{mi} water-stable microaggregates, WSA_{ma} water-stable macroaggregates

condensation ($Q^{4/6}{}_{HS}$: $r = -0.673$, $P \leq 0.01$; $Q^{4/6}{}_{HA}$: $r = -0.668$, $P \leq 0.01$) were associated with higher content of WSA_{mi} which means that in agricultural soils, the WSA_{mi} were stabilized mostly by C_{HA} with highly condensed and stabilized macromolecules, which is opposite to the WSA_{mi} in forest soils (Tables 4 and 5). This phenomenon can be explained as a consequence of cultivation, which destroys the original soil structure, breaks the macroaggregates to microaggregates and exposes physically protected labile organic material to microbial mineralization. Based on a hierarchical system of aggregation conceptualized by Tisdall and Oades (1982), primary particles forming microaggregates (cemented by persistent, aromatic humic material in association with amorphous Fe and Al and polyvalent metals) are bound together into macroaggregates by transient (microbial- and plant-derived polysaccharides) and temporary binding agents (roots and fungal hyphae). Pardo et al. (1997) found that intensive cultivation has led to a relative concentration of the colloidal fractions (humic acids and fulvic acids) in the microaggregates, accompanied by a selective depletion of the latter organic fraction.

Unlike microaggregates, the stabilization of WSA_{ma} in agricultural soil was associated with lower degree of humification (highly negative correlation with C_{HA}:C_{FA}) as well as with lower C_{HS} and C_{HA} condensation (positive correlations with $Q^{4/6}{}_{HS}$ and $Q^{4/6}{}_{HA}$), which is important difference in the stabilization of WSA_{ma} between studied agricultural and forest soils. Considering merely the humus fractions, the stabilization of WSA_{ma} in agricultural soils was provided mainly by C_{FA3}, while in forest soil, the stabilization of macroaggregates was provided with all fractions of C_{HA} and C_{FA}. Thus, the conversion of forest to farmland considerably changed the organic agents stabilizing WSA_{ma} and WSA_{mi}. This once again confirmed the known fact that organic matter is one of the most important agents of water-stable structure formation (Rajkai et al. 2015; Schacht and Marschner 2015). The fact that humic acids were not essential for macroaggregate stability in agricultural soil was confirmed by the negative correlations of WSA_{ma} with content of overall C_{HA}, as well as with C_{HA3} and C_{HA2} bound with clay, sesquioxides, and bivalent cations. In contrast, the stability of WSA_{ma} was highly positively influenced by C_{FA3}; therefore, we

Table 5 Correlation coefficients (r) between average values of soil organic matter parameters and individual size fractions of water-stable aggregates in agricultural soils

	WSA_{mi}	WSA_{ma}			
	Size fractions of water-stable aggregates in mm				
	< 0.25	> 5	5–3.15	3.15–1	1–0.25
C_{org}	-0.530^{*}	0.656^{**}	0.637^{*}	n.s.	n.s.
C_{HA}:C_{FA}	0.996^{***}	-0.997^{***}	-0.999^{***}	-0.985^{***}	-0.790^{***}
$Q^{4/6}{}_{HS}$	-0.673^{**}	0.549^{*}	0.570^{*}	0.736^{**}	0.967^{***}
$Q^{4/6}{}_{HA}$	-0.668^{**}	0.543^{*}	0.564^{*}	0.731^{**}	0.965^{***}
C_{HA1}	n.s.	n.s.	n.s.	n.s.	n.s.
C_{HA2}	0.901^{***}	-0.822^{***}	-0.836^{***}	-0.936^{***}	-0.992^{***}
C_{HA3}	0.589^{*}	-0.708^{**}	-0.690^{**}	-0.515^{*}	n.s.
ΣC_{HA}	0.687^{**}	-0.793^{**}	-0.777^{***}	-0.620^{*}	n.s.
C_{FA1a}	n.s.	-0.544^{*}	-0.523^{*}	n.s.	n.s.
C_{FA1}	n.s.	n.s.	n.s.	n.s.	0.591^{*}
C_{FA2}	0.612^{*}	n.s.	n.s.	-0.680^{**}	-0.944^{***}
C_{FA3}	-0.996^{***}	0.970^{***}	0.976^{***}	0.999^{***}	0.884^{***}
ΣC_{FA}	-0.547^{*}	n.s.	n.s.	0.619^{*}	0.914^{***}
C_{org} in WSA	n.s.	0.587^{*}	0.573^{*}	n.s.	n.s.
C_L in WSA	n.s.	n.s.	n.s.	n.s.	n.s.
C_{HWD} in WSA	n.s.	n.s.	n.s.	n.s.	n.s.

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $n = 15$

n.s. non-significant, C_{org} soil organic carbon, C_{HA}:C_{FA} the carbon of humic acids to carbon of fulvic acids ratio, $Q^{4/6}{}_{HS}$ color quotient of humic substances, $Q^{4/6}{}_{HA}$ color quotient of humic acids, C_{HA1} humic acids bound with monovalent cations, C_{HA2} humic acids bound with bivalent cations, C_{HA3} humic acids bound with the clay and sesquioxides, ΣC_{HA} total content of humic acids, C_{FA1a} free fulvic acids, C_{FA1} fulvic acids bound with monovalent cations, C_{FA2} fulvic acids bound with bivalent cations, C_{FA3} fulvic acids bound with the clay and sesquioxides, ΣC_{FA} total content of fulvic acids, C_{org} *in WSA* soil organic carbon in water-stable aggregates, C_L *in WSA* labile carbon in water-stable aggregates, C_{HWD} *in WSA* hot water-soluble carbon in water-stable aggregates, WSA_{mi} water-stable microaggregates, WSA_{ma} water-stable macroaggregates

consider that this humus fraction, bound with clay and sesquioxides, is a key to WSA_{ma} stability in the studied agricultural soils. Tisdall and Oades (1982) found the aggregation by SOM is favored by the presence of metal ions. Organic matter improves the aggregation due to their constituent fractions which stabilize soil aggregates by forming strong complexes with polyvalent metals (Al, Fe, Mn) adsorbed on clay. Consistent with our findings, Shujie et al. (2009) also noted that aggregate formation is provided particularly by fulvic acids and the association of aggregate classes > 2 and > 2–1 mm with SOM was weaker than with fulvic acids and that these aggregate classes had very weak association with humic acids and humins. Supporting our findings, Kotzé et al. (2016) observed that fulvic acids were significantly higher in the cultivated soils than in the virgin soils, which can be related to site-specific conditions, or to higher turnover of fulvic acids. Great importance of humic substances at aggregation process was confirmed by Guimaraes et al. (2013) who noted that humic and fulvic acid fractions can be rather used as indicators of soil quality than the whole SOM. Dou et al. (2013) studied the dynamics of newly formed humic acid and fulvic acid in aggregates after addition of the ^{14}C-labeled wheat residues for 60, 180, and 360 days incubation. The results showed that macroaggregates on newly formed $^{14}C_{HA}$ and $^{14}C_{FA}$ were higher than microaggregates; in the macroaggregates, the $^{14}C_{HA}$:$^{14}C_{FA}$ ratio decreased with the increase of incubation time and the formation of $^{14}C_{FA}$ was faster than $^{14}C_{HA}$, which the humic substance formation supported the lignin theory of Waksman. Since C_{FA} are generally less condensed than C_{HA}, they are more exposed to oxygen and micro-organisms and therefore more susceptible to mineralization leading to disintegration of soil macroaggregates (Stevenson 1982). Therefore, it is very important to sufficiently supply agricultural soils with organic manure. A higher content of C_L in WSA had a positive effect on WSA_{mi} formation in forest soils (Table 4). This was not observed, however, in agricultural soils (Table 5). On the other hand, in agricultural soils, C_{org} in WSA was positively correlated with WSA_{ma} > 3.15 mm. We infer that in agricultural soils, the stabilization of WSA_{ma} was associated with C_{FA3}, i.e., humified and more stable SOM as it has been shown in other studies (Šimanský et al. 2013; Šimanský et al. 2014).

4 Conclusions

Our results indicate that current soil management practices in agricultural soils based on intensive tillage or abandoned grassland are unsustainable. In forest soils, greater contents of macroaggregates than microaggregates were recorded as compared to agricultural soils. We conclude that in agricultural soils, the stabilization of water-stable macroaggregates was associated with humified, e.g., stable soil organic matter. The water-stable macroaggregate content was highly positively influenced mainly by fulvic acids bound with clay and sesquioxides; therefore, we consider this humus fraction to be a key to macroaggregate stability in the studied agricultural soils. On the other side, all fractions of humic and fulvic acids participated on the formation of water-stable macroaggregates in forest soil, which was a major difference in organic stabilization agents between studied forest and agricultural soils. Another considerable difference is that water-stable microaggregates in agricultural soils were stabilized primarily with humic acids and in forest soils by fulvic acids. Moreover, in forest soils, a higher content of labile carbon in water-stable aggregates had a positive effect on formation of water-stable microaggregates. The observed changes in individual size classes of water-stable aggregates and interactions between soil organic matter, particle-size distribution, and water-stable aggregates have a negative impact on soil fertility and thereby endanger agricultural sustainability. The promotion of a stable soil structure is one of the ways of restoring eroded and otherwise degraded soils.

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