Humus Substances and Soil Aggregates in the Soils with Different Texture

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Abstract

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Humus substances (HS) influence the incorporation of carbon into soil aggregates in many ways. In this study the influence of HS and their fractions in the soil on the proportions of carbon (total organic, labile, non-labile) in water-resistant macro-aggregates (WSA) and differences between the amount of carbon in WSA in coarse-grained (CGS) and fine-grained (FGS) soils with dependence on the proportions of HS in the soil were determined. The experiment included three soils (Haplic Chernozem, Haplic Luvisol, Eutric Cambisol), each of them with two different soil textures (CGS, FGS) from four ecosystems (forest, meadow, urban, and agro-ecosystem). In CGS, higher proportions (52 and 50%) of smaller (< 1 mm) dry-sieved macro-aggregates (DSA) and also WSA were determined, while in FGS, higher proportions (51 and 53%) of larger DSA (> 7 mm) and WSA (> 2 mm) were detected. A negative correlation was recorded between the content of organic carbon in the fractions of WSA and the amount of extracted humic acids (HA) in CGS, and fulvic acids (FA) in FGS. In CGS, the correlation between the carbon content in WSA and HA bound with Ca^{2+} and Mg^{2+}, which forms humates (HA2), was negative. In FGS, a negative correlation was recorded between the carbon content in WSA and free aggressive FA (FA1a) and free FA and those, which are bound with monovalent cations and mobile R_2O_3 (FA1) in the soil. In the case of FA1a, a negative correlation was recorded in FGS and also in CGS, however this influence was more marked in CGS than in FGS (by about 21% higher correlation). In CGS, the influence of HA and FA in soil on the content of labile carbon in aggregates was stronger than in FGS. In CGS, a higher proportion of carbon in aggregates was detected in the case of lower stability of HS and HA and, on the contrary, in FGS, a higher content of carbon in aggregates was detected in the case of their higher stability.

Keywords: fulvic acids; humic acids; macro-aggregates; organic carbon; soil texture

The accumulation and decomposition of soil organic carbon are in a close relation with molecular characteristics of soil organic matter (SOM) (Spaccini *et al.* 2002), which is important in the stabilization of macro-aggregates (Helfrich *et al.* 2008). Soil aggregation is closely linked to different fractions of SOM, mainly with polysaccharides, humic acids

(HA), and fulvic acids (FA) (Hayes & Swift 1990). The positive influence of humus substances (HS) on the quality of soil and soil aggregates is evident (Bartlová *et al.* 2015). The cementation effect of HA has long been considered a major factor in the formation of soil structure, which is very important especially in sandy soils (Tan 2003). The fine frac-

tions (clay, silt) are in a positive correlation with total organic carbon (TOC) and the coarse fractions (sand) in a negative (Burke *et al.* 1989; Bronson *et al.* 2004). A significant part of clay minerals is in the linkage with a large amount of organic substances (Jastrow 1996), which can be stabilized through the adsorption on the mineral surfaces (Baldock & Skjemstad 2000), bridges of polyvalent cations (Muneer & Oades 1989), binding into the interlayers of clay minerals (Kleber *et al.* 2007). The stabilization of SOM is mainly controlled by the organo-mineral association (Cai *et al.* 2016) and the iron-oxides and hydroxides. The content and quality of HS strongly influence the total and labile trace elements content in the soil (Karabcová *et al.* 2015) that can play the role of possible glue agents. HS are one of the most important fractions of SOM (Vergnoux *et al.* 2011), which may also through its functional groups enter into interactions with metal ions to form complexes. In terms of a long term stability of soil aggregates, their humified and hydrophobic components are mainly important (Sodhi *et al.* 2009). The objectives of this study were as follows: (*i*) to assess the influence of HS and their fractions in the soil on the proportions of carbon (TOC, labile, non-labile) in water-resistant macro-aggregates (WSA), (*ii*) to determine the differences between the amount of carbon in WSA in coarse-grained (CGS) and fine-grained (FGS) soils with dependence on the proportions of HS in the soil.

MATERIAL AND METHODS

Characteristics of the territory. The study areas are located in different parts of Slovakia. Haplic Chernozem comes from the localities Pata (48°16'N, 17°49'E) and Voderady (48°16'N, 17°34'E) and one of the Haplic Luvisols comes from the locality Veľké Zálužie (48°18'N, 17°57'E), which are situated on the northern border of the Danube Basin. Geological structure is characterized by Neogene strata, which are covered with younger Quaternary rocks. The other Haplic Luvisol comes from the locality

Table 1. Differences in the soil texture (%) in the coarse-grained (CGS) and fine-grained soils (FGS) analyzed

	Sand	Silt	Clay
CGS	48.50[b]	41.71[a]	9.79[a]
FGS	18.97[a]	53.69[b]	27.34[b]

[ab]statistically significant differences (*P* < 0.05, Tukey's test)

Table 2. Differences in the fractional composition of dry-sieved macro-aggregates (%) in the coarse-grained (CGS) and fine-grained soils (FGS) analyzed

	> 7	5–7	3–5	1–3	0.5–1	0.25–0.5
	(mm)					
CGS	17.38[a]	14.02[a]	16.86[a]	18.80[a]	13.44[b]	7.64[b]
FGS	35.27[b]	15.07[a]	17.29[a]	22.04[a]	7.59[a]	2.50[a]

[ab]statistically significant differences (*P* < 0.05, Tukey's test)

Včelince (48°23'N, 20°19'E) situated in the Southern Slovak Basin. Geological structure is characterized by Mesozoic gemerids. Eutric Cambisol comes from the locality Selce (48°46'N, 19°12'E), which is situated at the slopes of the Staré Hory Mountains. Geological structure is characterized by the core mountains of the Central Western Carpathians, which are covered with shales, sandstones, and dolomites. The other Eutric Cambisol comes from the locality Prietrž (48°40'N, 17°26'E), which is situated at the Myjava Hills. Geological structure is characterized by the Carpathian flysch.

Experimental details. The experiment included three soil types (Haplic Chernozem, Haplic Luvisol, Eutric Cambisol) (IUSS Working Group WRB 2006), each of them with two different soil textures (CGS, FGS) (Table 1), derived from four types of ecosystems (forest, meadow, urban, and agro-ecosystem). These are the soils of lowlands and uplands, which have the largest proportion in Slovakia and are intensively agriculturally used. The forest ecosystems were natural forests with human control, the meadow ecosystems were created by man 30 years ago, and the urban ecosystem soils were derived from urban landscape (grasses in a town influenced by human activities). The fields in the agro-ecosystems were managed by different farms under real production conditions.

Soil samples and analytical methods used. The soil samples were collected in three replicates to a depth of 0.30 m, and dried at a constant room temperature of 25 ± 2°C. For determining the chemical

Table 3. Differences in the fractional composition of water-resistant macro-aggregates (%) in the coarse-grained (CGS) and fine-grained soils (FGS) analyzed

	> 5	3–5	2–3	1–2	0.5–1	0.25–0.5
	(mm)					
CGS	6.00[a]	7.04[a]	10.46[a]	13.53[a]	20.84[b]	20.68[b]
FGS	17.40[b]	16.36[b]	15.93[b]	15.85[a]	13.33[a]	7.54[a]

[ab]statistically significant differences (*P* < 0.05, Tukey's test)

Table 4. Differences in the contents of organic carbon (mg/kg) in the coarse-grained (CGS) and fine-grained soils (FGS) analyzed

	TOC	C_L	C_{NL}
CGS	15 684[a]	2 215[a]	13 468[a]
FGS	19 047[a]	2 216[a]	16 831[a]

TOC – total organic carbon, C_L – labile carbon, C_{NL} – non-labile carbon; [a]statistically significant differences ($P < 0.05$, Tukey's test)

Table 6. Differences in the content of total organic carbon in fractions of water-resistant macro-aggregates (mg/kg) in the coarse-grained (CGS) and fine-grained soils (FGS) analyzed

	> 5	3–5	2–3	1–2	0.5–1	0.25–0.5
CGS	18 682[a]	18 303[a]	18 342[a]	18 919[a]	17 120[a]	15 501[a]
FGS	21 076[a]	20 313[a]	20 103[a]	20 244[a]	19 842[a]	18 820[a]

[a]statistically significant differences ($P < 0.05$, Tukey's test)

properties, the samples were ground. To determine the fractions of soil aggregates (Tables 2 and 3), the soil samples were divided by the sieve (dry and wet sieve) to size fractions of the net aggregates (Sarkar & Haldar 2005). The particle size distribution was determined after dissolution of $CaCO_3$ with 2 mol/dm³ HCl and oxidation of the organic matter with 30% H_2O_2. After repeated washing, the samples were dispersed using $Na(PO_3)_6$. Silt, sand, and clay fractions were determined according to the pipette method (van Reeuwijk 2002). In the soil (Table 4) and soil aggregates (Tables 5 and 6), the TOC by wet combustion (Orlov & Grišina 1981) and labile carbon (C_L) by $KMnO_4$ oxidation (Loginov *et al.* 1987) were determined. The fractions of HS (Table 7) – free HA and HA bound with monovalent cations and mobile R_2O_3 (HA1), HA bound with Ca^{2+} and Mg^{2+} which forms humates (HA2), HA bound with mineral

components and stabile R_2O_3 (HA3), free aggressive FA (FA1a), free FA and FA bound with monovalent cations and mobile R_2O_3 (FA1), FA bound with Ca^{2+} and Mg^{2+} which forms fulvates (FA2), FA bound with mineral components and stabile R_2O_3 (FA3) were determined according to the method of Ponomareva and Plotnikova (1975).

The obtained data were analyzed using Statgraphic Plus statistical software (Ver. 4, 1994). A multifactor ANOVA model was used for individual treatment comparisons at $P < 0.05$, with separation of the means by Tukey's multiple-range test. Correlation analysis (Pearson's correlation coefficient) was used to determine the relationships between the HS in the soils and carbon in the soil aggregates. Significant correlation coefficients were tested at $P < 0.05$ and $P < 0.01$.

RESULTS AND DISCUSSION

The proportion of aggregate fractions and soil texture. In this experiment, the relation of stabile organic substances like HS in the soil and soil aggregates was influenced mainly by textural composition, soil genesis, and finally by ecosystem. The proportion of DSA and WSA fractions was different in CGS and FGS. Larger DSA (> 7 mm) and WSA (> 2 mm) had higher proportion in FGS and smaller DSA (< 1 mm)

Table 5. Differences in the content of total organic carbon in fractions of dry-sieved macro-aggregates (mg/kg) in the coarse-grained (CGS) and fine-grained soils (FGS) analyzed

	> 7	5–7	3–5	1–3	0.5–1	0.25–0.5
CGS	17 511[a]	17 763[a]	17 706[a]	18 558[a]	19 031[a]	16 578[a]
FGS	17 899[a]	18 700[a]	19 155[a]	19 844[a]	20 882[a]	20 911[a]

[a]statistically significant differences ($P < 0.05$, Tukey's test)

Table 7. Differences in the fractional composition of humus substances (%) in the coarse-grained (CGS) and fine-grained soils (FGS) analyzed

	HA1	HA2	HA3	ΣHA	FA1a	FA1	FA2	FA3	ΣFA
CGS	8.30[b]	14.56[b]	11.87[a]	34.63[a]	6.97[b]	13.48[b]	8.62[a]	7.99[a]	37.06[a]
FGS	6.42[a]	10.78[a]	15.70[b]	32.90[a]	4.49[a]	4.88[a]	10.65[a]	10.08[b]	29.77[b]

HA1 – humic substances free and those bound with monovalent cations and mobile R_2O_3, HA2 – bound with Ca^{2+}, HA3 – bound with mineral components and stabile R_2O_3, ΣHA – sum of humic acids, FA1a – free aggressive fulvic acids, FA1 – bound with monovalent cations and mobile R_2O_3, FA2 – bound with Ca^{2+}, FA3 – bound with mineral components and stabile R_2O_3, ΣFA – sum of fulvic acids; [ab]statistically significant differences ($P < 0.05$, Tukey's test)

and WSA (< 1 mm) in CGS (Tables 2 and 3). In FGS, larger aggregates are formed more easily, because they have a higher content of clay, which has a large specific surface and a number of available binding positions that are important in the formation of organo-mineral components (Eustehues *et al.* 2003). In CGS, the content of organic substances playing a function of glue agent is lower, so that the aggregates are more susceptible to disruption, therefore there is a lower proportion of larger aggregates and a higher proportion of smaller aggregates. The proportions of DSA (1–5 mm) and WSA (1–2 mm) fractions with a more optimal size were relatively balanced in CGS and FGS (Tables 2 and 3).

Quantity of humus substances in the soil and carbon content in the aggregates. HS not only participate on the formation of stabile macro-aggregates (Tisdall & Oades 1982), but significantly influence the contents of carbon in the aggregates fractions. A negative correlation was recorded between the content of organic carbon in the fractions of WSA and the amount of extracted HA in CGS and FA in FGS (Table 8). In CGS, the formation of aggregates is greatly limited due to a high content of sand fraction, which does not provide sufficient binding positions for the formation of organo-mineral complexes (Creamer *et al.* 2013). There are mainly organic substances with dominant aliphatic components (Jindaluang *et al.* 2013) and organic substances that subject to more intensive oxidation, at which the pH decreases, which also results in the removing of further potential glue agents, that are bi- and tri-valent cations. As FA are soluble under a whole range of pH, they migrate into the lower parts of the soil profile (Perminova *et al.* 2005), and so in CGS, in relation to the aggregates formation they play rather a role of an indirect factor (decreasing of pH) and their direct influence is not more markedly reflected. FA have also a higher acidity than HA, because in comparison to them FA contain more functional groups with the acidic reaction (-COOH, -C=O, -OH) (Stevenson 1994). FA are an important source of H^+ and in FGS they act also destructively on the inorganic components, particularly on the clay minerals, thereby many bonds in aggregates are disrupted and the aggregates break down. Therefore, at a higher proportion of FA in the soil, a lower content of carbon (mainly non-labile (C_{NL})) in the aggregates, in which it was originally stabilized, was reflected (Table 8). On the contrary, the influence of HA was reflected in CGS. Due to the decrease of pH in the

process of the organic substances decomposition, with the present mobilization of bi- and tri-valent cations, causes the precipitation of HA, and the part of available binding positions is blocking (Brady 1990) that decrease their properties as glue agents. Thus it indirectly inhibits also the incorporation of labile components into soil aggregates that are not able to create stabile bonds with sand. Therefore in CGS, at a higher proportion of HA in the soil, a lower content of not only labile, but also stabile components in the soil aggregates was reflected (Table 8).

Quality of humus substances in the soil and carbon content in the aggregates. The previous is supported by different correlations between the content of carbon in the aggregates and stability of HS and HA. In the case of CGS, a positive correlation and in the case of FGS a negative correlation was detected between the contents of labile carbon (C_L) and non-labile carbon (C_{NL}) in the aggregates and coloured quotients of HS (Q_{HS}) and HA (Q_{HA}) (Table 8). In CGS, a higher carbon content in the aggregates was at a lower stability of HS and HA. In CGS, fine roots and fungal hyphae significantly participate in the stabilization of macro-aggregates, therefore these aggregates are richer in C_L and there is also a smaller amount of components in the environment that would contribute to stabilization of HS. Moreover, if HA are less stabilized and younger and also if there is a sufficient amount of easily available radicals in the environment, a more intensive recovery of their peripheral parts occurs. On the contrary, in FGS, at a higher stability of HA the carbon content in the aggregates is higher, too. Here the HS create bonds with clay minerals, they are not only adsorbed on their surfaces, but also can penetrate into clay interlayers (Kleber *et al.* 2007). In FGS, aromatic carbon components are dominant in organic structures (Jindaluang *et al.* 2013). HA themselves can also be a part of the aggregates, so at a higher stability of HA there is also a higher carbon content in the aggregates.

In CGS, differences in the impact of various fractions of HA were recorded, too. The carbon content in the aggregates was in a negative correlation with HA2 (Table 2). HA2 is the fraction of HA that is bound with bivalent cations, so under the influence of Ca^{2+} they can be precipitated from the soil solution.

In the case of FGS, the correlation with HA2 was not recorded because in these soils due to a higher proportion of clay minerals there can act more mechanisms of aggregation, which overlap each other, thus the impact of this fraction was not more

Table 8. Correlation between carbon in fractions of water-resistant macro-aggregates and parameters of humus substances in the soil

Soil texture	Aggregate fraction (mm)	HA2	FA1a	FA1	HS	HA	FA	Q_{HS}	Q_{HA}
Total organic carbon coarse-grained soils	2–3	−0.526**	−0.669**	ns	−0.671**	−0.526*	ns	0.536*	0.545*
	1–2	−0.542*	−0.691**	ns	−0.663**	−0.507*	ns	0.555**	0.573**
	0.5–1	−0.620**	−0.678**	ns	−0.630**	−0.492*	ns	0.641**	0.657**
	0.25–0.5	−0.600**	−0.661**	ns	−0.599**	ns	ns	0.620**	0.635**
fine-grained soils	> 5	ns	−0.555**	ns	ns	ns	−0.442*	−0.624**	−0.684**
	3–5	ns	−0.599**	−0.510*	ns	ns	−0.547*	−0.727**	−0.752**
	2–3	ns	−0.606**	−0.520*	ns	ns	−0.588**	−0.763**	−0.784**
	1–2	ns	−0.617**	−0.485*	ns	ns	−0.570**	−0.709**	−0.739**
	0.5–1	ns	−0.587**	−0.454*	ns	ns	−0.526*	−0.738**	−0765**
	0.25–0.5	ns	−0.617**	ns	ns	ns	−0.538*	−0.736**	−0.770**
Labile carbon coarse-grained soils	2–3	ns	−0.585**	ns	−0.559**	−0.690**	ns	ns	ns
	1–2	ns	−0.534*	ns	−0.487*	−0.704**	ns	0.551**	0.538*
	0.5–1	−0.563**	−0.605**	ns	−0.517*	−0.741**	ns	0.687**	0.677**
	0.25–0.5	−0.460*	−0.673**	ns	−0.545*	−0.555**	ns	0.594**	0.573**
fine-grained soils	> 5	ns	ns	ns	ns	ns	ns	ns	−0.516*
	3–5	ns	−0.447*	ns	ns	ns	ns	−0.542*	−0.598**
	2–3	ns	−0.500*	ns	ns	0.451*	−0.458*	−0.689**	−0.725**
	1–2	ns	ns	ns	ns	ns	ns	−0.550**	−0.606**
	0.5–1	ns	−0.494*	ns	ns	ns	ns	−0.693**	−0.745**
	0.25–0.5	ns	−0.502*	ns	ns	ns	ns	−0.689**	−0.751**
Non-labile carbon coarse-grained soils	2–3	−0.570**	−0.664**	ns	−0.671**	−0.482*	ns	0.544*	0.566**
	1–2	−0.560**	−0.698**	ns	−0.674**	−0.460*	ns	0.541*	0.563**
	0.5–1	−0.611**	−0.669**	ns	−0.627**	−0.446*	ns	0.618**	0.637**
	0.25–0.5	−0.615**	−0.647**	ns	−0.599**	ns	ns	0.614**	0.635**
fine-grained soils	> 5	ns	−0.570**	−0.441*	ns	ns	−0.467*	−0.643**	−0.693**
	3–5	ns	−0.612**	−0.514*	ns	ns	−0.568**	−0.743**	−0.759**
	2–3	ns	−0.613**	−0.533*	ns	0.439*	−0.599**	−0.761**	−0.779**
	1–2	ns	−0.637**	−0.503*	ns	ns	−0.598**	−0.717**	−0.740**
	0.5–1	ns	−0.591**	−0.469*	ns	0.433*	−0.533*	−0.729**	−0.752**
	0.25–0.5	ns	−0.624**	ns	ns	ns	−0.545*	−0.732**	−0.761**

HA2 – humic acids bound with Ca^{2+}; FA1a – free aggressive fulvic acids; FA1 – fulvic acids free and bound with mobile R_2O_3; HS – humus substances; HA – humic acids; FA – fulvic acids; Q_{HS} – coloured quotient of humus substances; Q_{HA} – coloured quotient of humic acids; **$P < 0.01$; *$P < 0.05$; ns – not significant

The values are presented as correlation coefficients (i.e. without units); the fractions processed (including units) were: fractions of macro-aggregates (%); total organic carbon, labile carbon, and non-labile carbon (mg/kg); fractions of humus substances (in % of total organic carbon)

pronounced. FGS, with a higher content of clay minerals, mainly smectites, are subjected also to higher volume changes (Dalal & Bridge 1996) and thus retain a higher amount of water. Water can serve also as a bridge between organic ligand and the clay micelle (Tan 2003).

On the other hand, in FGS, a negative correlation between the carbon content, mainly non-labile, in the aggregates and FA1a and FA1 in the soil was recorded. In the case of FA1a, a negative correlation was recorded in CGS, as well. FA are more acidic than HA, have substantially higher content of COOH groups and are an important source of H^+, moreover FA1a are a fraction of free aggressive FA. At a lower pH, a higher amount of carbon is in a labile form (Tobiašová 2011), resulting in a decreasing of the aggregate stability and releasing of carbon from them. This process is more marked in CGS than in FGS. But the influence of FA1 was recorded only in FGS and only in relation to C_{NL}. Both fractions, FA1a and FA1, are characterized by high acidity, but FA1 consists partly of free fulvic acids, although for the most part it consists of salts and complex compounds with mobile hydrated sesquioxides, mainly with aluminium (Kononova 1966). Monovalent cations, mainly Na^+, are a highly dispersive agent that directly causes disruption of aggregates (Batra *et al.* 1997). The result of this can be that at a higher proportion of FA1 in FGS the stability of carbon decreases through the disruption of aggregates, so that the C_{NL} content in the aggregates decreases. Overall, in CGS, the influence of HA and also FA on the C_L content in the aggregates was more marked than in FGS.

CONCLUSION

Humic acids were correlated mainly with the proportion of organic carbon, labile and non-labile, in the fractions of water-resistant macro-aggregates in the coarse-grained soils and the fulvic acids were correlated with their amounts in the fine-grained soils.

The influence of humic acids and fulvic acids in the soil more strongly affected the content of labile carbon in coarse-grained soils than in fine-grained soils. The higher was the stability of humus substances, the higher was the content of carbon in the aggregates in fined-grained soils and the lower in coarse-grained soils.

Fractions of humic acids and fulvic acids influenced the carbon content in water-resistant macro-aggregates in different way depending on the soil texture.

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