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Labile Forms of Carbon and Soil Aggregates

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Abstract

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Soil organic matter (SOM) plays an important role in the soil aggregation and *vice versa*, its incorporation into the soil aggregates is one of the mechanisms of soil organic carbon stabilization. In this study the influence of labile carbon fractions on the fractions of dry-sieved (DSA) and wet-sieved (WSA) macro-aggregates and the relationship between the content of total organic carbon (TOC) and its labile fractions in the soil and in the fractions of macro-aggregates were determined. The experiment included six soil types (Eutric Fluvisol, Mollic Fluvisol, Haplic Chernozem, Haplic Luvisol, Eutric Cambisol, Rendzic Leptosol) in four ecosystems (forest, meadow, urban, and agro-ecosystem). In the case of DSA, the contents of labile fractions of carbon, in particular cold water extractable organic carbon (CWEOC) and hot water extractable organic carbon (HWEOC), had a higher impact on the proportions of larger fractions of macro-aggregates (3–7 mm), while in the case of WSA, the impact of labile fractions of carbon, mainly labile carbon (C_L) oxidizable with $KMnO_4$, was higher on the proportions of smaller fractions of aggregates (0.25–1 mm). The WSA size fraction of 0.5–1 mm seems an important indicator of changes in the ecosystems and its amounts were in a negative correlation with C_L (r = –0.317; P < 0.05) and HWEOC (r = –0.356; P < 0.05). In the WSA and DSA size fractions 0.5–1 mm, the highest variability in the contents of TOC and C_L was recorded in the forest ecosystem > meadow ecosystem > urban ecosystem > agro-ecosystem. The higher were the inputs of organic substances into the soil, the greater was the variability in their incorporation into the soil aggregates. The influence of the content of TOC and its labile forms on their contents in the DSA and WSA was different, and the contents of TOC and C_L in the aggregates were more significantly affected by the C_L content than by water soluble carbon. In the case of WSA fractions, their carbon content was more affected in the 1–2 mm than in 0.5–1 mm fraction.

Keywords: ecosystem; labile carbon; macro-aggregates; soil type

Soil organic matter (SOM) and soil structure are in the mutual dynamic interaction and this relationship is influenced not only by soil properties (Martins *et al.* 2013; Wang *et al.* 2016), but also by the way of land use (Barreto *et al.* 2009; Wang *et al.* 2014). The positive influence of stabile components of SOM (like humus substances) on the quality of soil and soil aggregates is evident (Bartlová *et al.* 2015). However, according to Yang *et al.* (2012), the labile pools of carbon also significantly affect the quality of soil and moreover they can be used to monitor the changes in SOM in a shorter time period. The lability of SOM depends on its chemical recalcitrance and physical protection against microbial activity (Sil-

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Veria *et al.* 2008). The increase in SOM amount is associated with the increase of the amount of carbon-rich macro-aggregates (Six *et al.* 2002) and with the enrichment of water-resistant macro-aggregates with new SOM (Angers & Giroux 1996). Its components are incorporated into various levels of the soil aggregates hierarchy, from the first formation of basic organo-mineral complexes to stabilizing of larger aggregates (Six *et al.* 2004). Labile fractions include various components of organic matter in soil, which are characterized by a short turnover. Cold (CWEOC) and hot (HWEOC) water extractable organic carbons are the most active pools of SOM and play an important role in the global carbon turnover (Bu *et al.* 2011) and are a part of dissolved organic carbon (Zsolnay 2003; Fernández-Romero *et al.* 2016). Carbon that is oxidizable with $KMnO_4$ (C_L) includes all organic components that are easily oxidizable with $KMnO_4$, including the labile humus material and polysaccharides (Conteh *et al.* 1999).

The objectives of this study were (*i*) to assess the influence of labile carbon fractions (CWEOC, HWEOC, and C_L) on the proportions of dry-sieved (DSA) and wet-sieved (WSA) macro-aggregates, (*ii*) to determine the relationship between the content of total organic carbon (TOC) and its labile fractions (CWEOC, HWEOC, and C_L) in the soil and in the fractions of macro-aggregates (DSA, WSA).

MATERIAL AND METHODS

Characteristics of the territory. The studied areas are located in different parts of Slovakia. Mollic Fluvisol comes from the locality Horná Kráľová (48°24'N, 17°92'E) and Haplic Chernozem comes from the locality Pata (48°16'N, 17°49'E), which are situated on the northern border of the Danube Basin. Geological structure is characterized by neogene strata, which consist mainly of claystones, sandstones, and andesites covered with younger quaternary rocks represented by different fluvial and eolian sediments (Biely *et al.* 2002). Natural vegetation consists mostly of ash-oak-elm-alder forests, and along the river, there are willow-poplar forests and floodplain forests. In the elevated areas and dunes, xerophilic communities of oak-elm forests are dominant (Korec *et al.* 1997). Eutric Fluvisol and Haplic Luvisol come from the locality Vráble (48°24'N, 18°31'E), which is situated in the Danube Lowland, in the unit of the Danube Plain, namely in the Nitra Upland. Geological structure is characterized by neogene sediments – loess and loamy loess. Neogene bedrock is formed by lake and brackish sediments (clays, gravels, and sands) (Biely *et al.* 2002). In the lower parts there are oak forests and higher beech forests dominate. Rendzic Leptosol comes from the locality Turá Lúka (48°44'N, 17°32'E), which is situated at the eastern foot of the White Carpathians, in the Myjava Upland, in the valley of the river Myjava. Geological structure is characterized by the limestones and marls of the klippen belt, chalk clay-marl layers and neogene conglomerates, sandstones, and sands. In the forests, oak and hornbeam dominate, with beech in higher parts and conifers on dolomites and limestones (Korec *et al.* 1997). Eutric Cambisol comes from the locality Spišská Belá (49°11'N, 20°27'E), which is situated in the northern part of the Poprad Basin. Geological structure is characterized by the “core mountains” of the Central Western Carpathians. This part of the mountains is composed of mica schists to gneisses, locally early palaeozoic phyllites, which are covered with deluvial sediments (Biely *et al.* 2002). In the forests, spruce dominates.

Experimental design. The experiment included four types of ecosystems representing different land use (forest, meadow, urban, and agro-ecosystems) of six soil types (Eutric Fluvisol, Mollic Fluvisol, Haplic Chernozem, Haplic Luvisol, Eutric Cambisol, and Rendzic Leptosol) (WRB 2006) most frequent in Slovak lowlands and uplands and intensively agriculturally used. The forest ecosystems are natural forests with human control, the meadow ecosystems were established by man 30 years ago, the urban ecosystems are represented by soils of urban landscape (grasses in a town influenced by human activities), and the fields in agro-ecosystems are located at different farms under real production conditions.

Soil samples and analytical methods used. The soil samples for chemical and physical analysis were collected in three replicates to a depth of 0.30 m, and dried under a constant room temperature of 25 ± 2°C. Basic chemical and physical properties of the soils are characterized in the Table 1. For conducting the chemical analysis, the soil samples were ground. To determine the fractions of soil aggregates, the soil samples were divided by a 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 HCl/dm^3 and oxidation of the organic matter with 30% H_2O_2. After repeated washing, samples were dispersed using $Na(PO_3)_6$. Silt, sand, and clay fractions were determined according

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to the pipette method (van Reeuwijk 2002). In the soil and soil macro-aggregates (dry and wet sieve), the TOC by wet combustion (Orlov & Grišina 1981) and C_L by $KMnO_4$ oxidation (Loginov *et al.* 1987) were determined. In the soil, CWEOC and HWEOC were determined according to the method by Ghani *et al.* (2003) with the final determination of organic carbon by wet combustion (Orlov & Grišina 1981). The pH of the soil was potentiometrically measured in a supernatant suspension of a 1 : 2.5 soil : liquid mixture. The liquid is 1 mol KCl/dm^3 (pH_{KCl}) (van Reeuwijk 2002); total exchange basis (T) was determined according to Soil Survey Staff (2014).

The obtained data were analyzed using Statgraphic Plus statistical software (Ver. 4, 1994). A multi-factor 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 was used to determine the relationships between the chemical properties and the parameters of soil structure stability. Significant correlation coefficients were tested at $P < 0.05$ and $P < 0.01$.

RESULTS AND DISCUSSION

Labile fractions of carbon and the proportion of soil aggregate fractions. The proportion of fractions of soil aggregates is influenced not only by the amount of carbon, but also its stability and/or lability. The relationships between the individual fractions

Table 1. Basic chemical and physical properties of soils in four different ecosystems

Soil type	Ecosystem	pH	TOC (g/kg)	T (mmol/kg)	Clay	Silt	Sand
						(%)	
Haplic Chernozem	FE	6.06	21.65	347.20	14.61	42.34	43.05
	ME	6.46	20.13	389.90	15.50	39.01	45.49
	UE	6.54	18.22	399.17	12.22	49.75	38.03
	AE	6.39	17.60	330.20	17.34	50.13	32.53
Mollic Fluvisol	FE	7.14	20.95	377.29	54.43	35.13	10.44
	ME	7.39	14.96	399.21	54.64	31.20	14.16
	UE	7.50	17.66	399.54	49.11	32.33	18.56
	AE	7.07	11.44	347.93	46.68	36.78	16.54
Eutric Fluvisol	FE	6.33	19.85	353.20	11.17	70.55	18.28
	ME	6.75	17.11	371.49	11.49	72.46	16.05
	UE	6.78	15.11	322.78	10.74	73.65	15.61
	AE	6.18	11.29	305.91	12.52	75.43	12.05
Haplic Luvisol	FE	5.51	11.07	346.38	16.68	73.87	9.45
	ME	5.87	8.63	321.44	17.47	73.68	8.85
	UE	6.17	9.23	353.32	11.74	78.93	9.33
	AE	6.06	8.09	320.12	17.66	72.45	9.89
Rendzic Leptosol	FE	7.07	31.86	408.74	11.48	69.25	19.27
	ME	7.33	23.52	470.89	11.00	74.05	14.95
	UE	7.37	20.07	468.66	10.64	72.75	16.61
	AE	7.16	13.40	421.75	12.14	68.54	19.32
Eutric Cambisol	FE	5.71	40.25	259.53	12.54	51.50	35.96
	ME	5.99	33.92	283.63	10.11	55.39	34.50
	UE	6.60	21.58	241.23	7.43	48.84	43.73
	AE	6.21	19.10	258.63	8.02	49.42	42.38

FE – forest ecosystem; ME – meadow ecosystem; UE – urban ecosystem; AE – agro-ecosystem; TOC – oxidizable organic carbon; T – total exchange bases

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of DSA and WSA and individual fractions of labile carbon are different (Table 2). In the case of the proportion of smaller fractions of WSA (0.25–1.0 mm), a negative correlation between these fractions and the content of carbon that is oxidizable with $KMnO_4$ (C_L) were recorded, and in the case of WSA (0.5–1.0 mm) also with HWEOC. A deterioration of the soil structure condition is reflected in the increased proportion of the smallest fractions of soil aggregates (Whalen & Chang 2002). These smallest fractions of macro-aggregates contain the lowest amounts of carbon (Sohi *et al.* 2001), however more stabilized than in the larger fractions. In the case of larger DSA fractions (3–7 mm), a positive correlation between these fractions and water extractable carbon was recorded. Larger fractions of aggregates contain higher amount of carbon, however subjected to more intensive mineralization (Six *et al.* 2000; Wang *et al.* 2014). One of the reasons is a higher proportion of more labile fractions of carbon; the second is greater access of the pores for soil microorganisms that participate in these transformation processes. According to Ranjard and Richaume (2001), the limitation of decomposers access to organic substances is a key factor in the physical stabilization of carbon. At the beginning of the carbon accumulation, it decides about the carbon next stabilization in DSA and hence its subsequent content in WSA. The tendency was similar when comparing within a set of six soil types and also in one soil type (Table 2). Water-resistant macro-aggregates of the 0.5–3.0 mm size fraction are agronomically the most valuable aggregates. However, it was revealed that a lower limit (0.5–1.0 mm) does not correspond to optimal condition of the soil structure. Many soils with deteriorated soil structure exhibited an increased content of macro-aggregates of 0.5–1.0 mm size fraction. On the contrary, this fraction seems to be an important indicator of changes in the ecosystems.

If we compare the contents of the 0.5–1.0 mm size fraction of WSA in individual ecosystems within the Haplic Luvisol soil type (Figure 1a), the highest proportion of this fraction was in the agro-ecosystem. Emadi *et al.* (2009) also reported that in the ploughed soil a higher proportion of micro-aggregates and small macro-aggregates remains. In the case of other ecosystems, the contents of the 0.5–1.0 mm size fraction were in a balance. If we compare the contents of the 0.5–1.0 mm size fraction in the individual ecosystems in the set of different soils (Figure 1b), the tendency is the same, but the differences between the ecosystems are more significant. The lowest content of this fraction was in the meadow ecosystem. In this ecosystem, the root system has a high impact on the

Table 2. Dependence between the fractions of dry-sieved (DSA) and wet-sieved (WSA) macro-aggregates and carbon in the set of six soil types and Haplic Luvisols

	Six soil types				Haplic Luvisols			
	TOC	C_L	CWEOC	HWEOC	TOC	C_L	CWEOC	HWEOC
DSA (mm)								
> 7	–0.242	–0.047	–0.314*	–0.309*	–0.358*	0.179	–0.032	–0.295
5–7	0.343*	0.202	0.435**	0.323*	0.162	0.052	0.544**	0.336
3–5	0.445**	0.346*	0.517**	0.543**	0.483**	0.180	0.313	0.419*
1–3	0.267	0.142	0.244	0.289	0.560**	0.109	–0.011	0.263
0.5–1.0	–0.049	–0.173	–0.074	–0.099	–0.048	–0.216	–0.222	–0.080
0.25–0.50	–0.140	–0.297	–0.124	–0.121	–0.264	–0.292	–0.138	–0.052
WSA (mm)								
> 5	0.081	0.287	–0.010	0.154	0.094	0.399*	0.226	0.153
3–5	0.303	0.445**	0.212	0.359*	0.223	0.255	0.004	0.095
2–3	0.393**	0.387*	0.259	0.279	0.419*	0.297	–0.187	0.172
1–2	0.155	–0.017	0.208	0.128	0.269	0.151	–0.223	0.124
0.5–1.0	–0.247	–0.368*	–0.098	–0.307*	–0.300	–0.317*	–0.219	–0.356*
0.25–0.50	–0.305*	–0.479**	–0.176	–0.340*	–0.318*	–0.378*	0.120	–0.120

TOC – total organic carbon; C_L – labile carbon; CWEOC – cold water extractable organic carbon; HWEOC – hot water extractable organic carbon; ***$P < 0.01$; *$P < 0.05$

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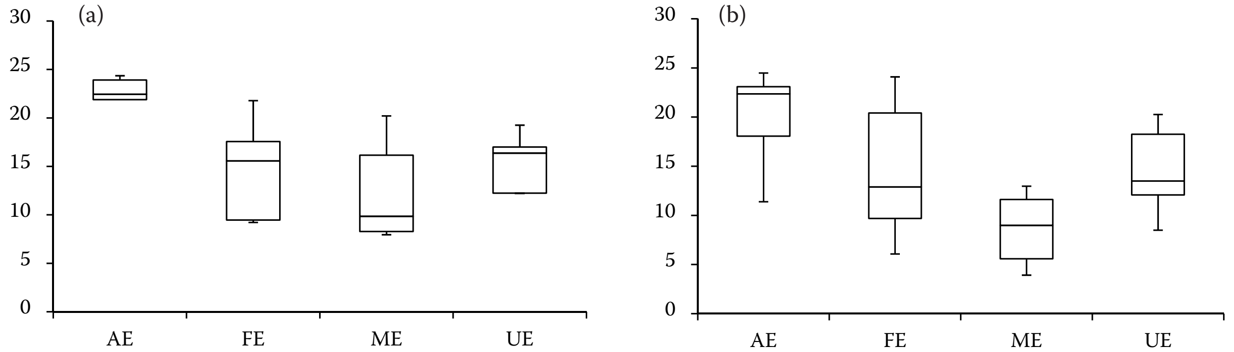

Figure 1. Differences (in %) in the 0.5–1.0 mm fraction of water-resistant macro-aggregates (WSA) in the ecosystems in a set of Haplic Luvisols (a) and of soil types (b)
AE – agro-ecosystem, FE – forest ecosystem, ME – meadow ecosystem, UE – urban ecosystem

aggregation (Tisdall & Oades 1982) that may subject the mechanical linking of these smaller aggregates. Moreover, according to Verchot *et al.* (2011), the polysaccharides coming from the microbial activity play a crucial role in the formation of stabile micro-aggregates and the carboxyl carbon supports in turn the stabilization through the occlusion. In addition to the dominance of more labile forms of carbon in the meadow ecosystem, there are also drier conditions in the root zone. Drying influences the stabilization of organic matter and also the formation of stronger linkages with soil mineral components.

More significant differences in the proportion of WSA of the 0.5–1.0 mm size fraction between the soil types were not recorded (Figure 2). Of the studied soil types, this fraction showed a more significantly lower proportion only in Rendzic Leptosol, in which also WSA of the > 5 mm size fraction had the highest proportion. The process of aggregation in Rendzic Leptosol compared to other soil types is different. This soil contains free calcium and in the case of skeletal Rendzic Letosols also drought plays an important role. Such conditions were during most of the year also in the meadow ecosystem, in which the lowest content of WSA of the 0.5–1 mm size fraction was also recorded. The drying may contribute to the formation of linkages between the organic molecules and mineral surfaces (Denef *et al.* 2002). However, in the case of meadow ecosystem, the lowest content of this fraction was a result of higher contents of the labile forms of carbon, so the influence was direct. In the case of Rendzic Leptosol, the influence of labile forms of carbon was indirect. A greater part of SOM was bonded primarily in the larger aggregates, which had, in this case, significantly higher proportion. The strength of their linkages with the mineral components is also higher, which limits the disruption and the proportion of smaller fractions of aggregates. A significant role in the aggregation is played by exchangeable Ca^{2+}, which supports the uptake and retention of water-soluble carbon (Setia *et al.* 2013).

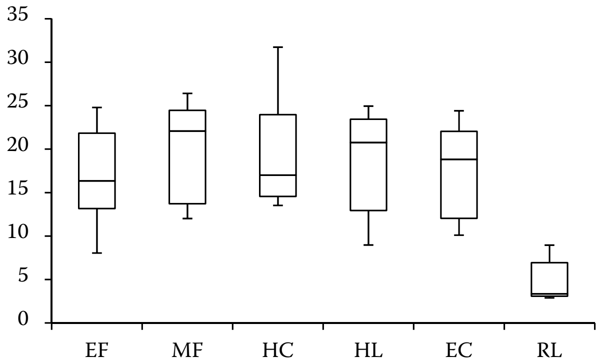

Figure 2. Differences (in %) in the 0.5–1.0 mm fraction of water-resistant macro-aggregates (WSA) in the set of soil types
EF – Eutric Fluvisol, MF – Mollic Fluvisol, HC – Haplic Chernozem, EC – Eutric Cambisol, RL – Rendzic Leptosol

Labile fractions of carbon and their proportion in the fractions of soil aggregates. Carbon content and its forms in the soil also influence its contents in the fractions of soil aggregates. In the WSA and DSA of the 0.5–1.0 mm size fraction, the highest variability in their contents was observed in the forest ecosystem. In this ecosystem, the highest inputs of carbon into the soil are reflected, and at the same time the highest variability of the forest communities, which results from the highest differences in the quality of organic substances inputs into the soil. The litter composition in coniferous and deciduous forests differs, or also the variability of the forest floor. The variability in carbon contents was more significant in the case of C_L (Figure 3) than

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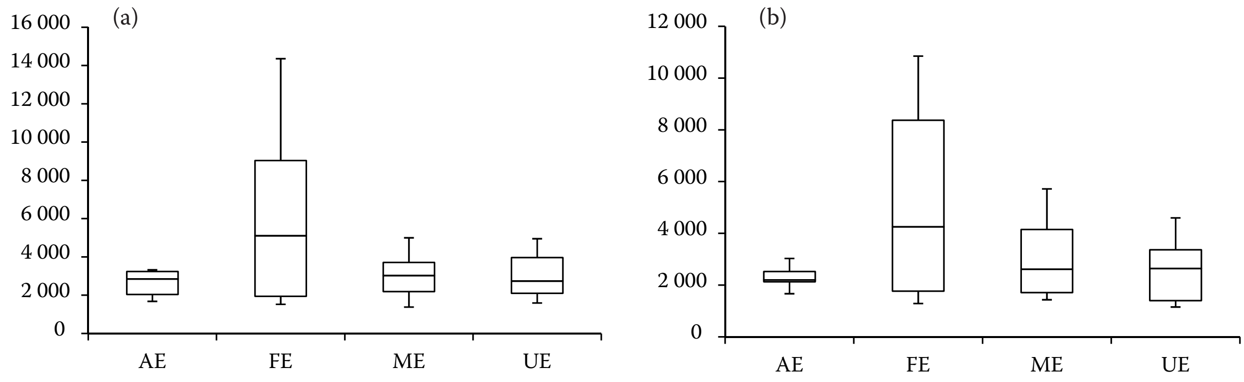

Figure 3. Labile carbon (C_L, mg/kg) in the 0.5–1.0 mm fraction of dry-sieved macro-aggregates (a) and of water-resistant macro-aggregates (b)
AE – agro-ecosystem, FE – forest ecosystem, ME – meadow ecosystem, UE – urban ecosystem

in TOC (Figure 4). The forest ecosystem was followed by meadow and urban ecosystems with grass vegetation cover. Organic inputs in these ecosystems were influenced by the inputs from aboveground biomass, left in place after mowing. The variability in the agro-ecosystem was clearly the lowest, with the lowest inputs into the soil. From the above-given it follows that the higher were the inputs of organic substances, the greater was the variability in their incorporation into the soil aggregates.

TOC content was in a direct relationship with the content of individual labile fractions. Yang *et al.* (2012) recorded a high positive correlation between TOC and C_L (r = 0.901; P < 0.01) in soil and Xue *et al.* (2013) also between TOC and HWEOC. In this case, a positive correlation between TOC and C_L in soil aggregates with the contents of all studied labile fractions of carbon (C_L, CWEOC, HWEOC) in soil was recorded (Table 3). The contents of TOC and its labile fractions in soil significantly influence the content of TOC in the soil aggregates sizing up to 7 mm. The influence of TOC and C_L in soil on the content of TOC in DSA increased with a reduction of the size fraction (r = –0.900; P < 0.05), and in the case of the influence of HWEOC in soil on TOC in DSA increased with the size fraction enlarging (r = –0.815; P < 0.10).

CONCLUSION

In the case of DSA, the contents of labile fractions of carbon, in particular CWEOC and HWEOC, had a higher impact on the proportions of larger fractions of macro-aggregates (3–7 mm), while in the case of WSA, the impact of labile fractions of carbon, mainly C_L, was higher on the proportions of smaller fractions of aggregates (0.25–1.00 mm).

WSA size fraction of 0.5–1.0 mm seems to be an important indicator of changes in the ecosystems and its amount was in negative correlation with C_L and HWEOC, thus its lower proportion will be in the ecosystems with higher sources of less stabilized organic matter, as is confirmed by the lowest content

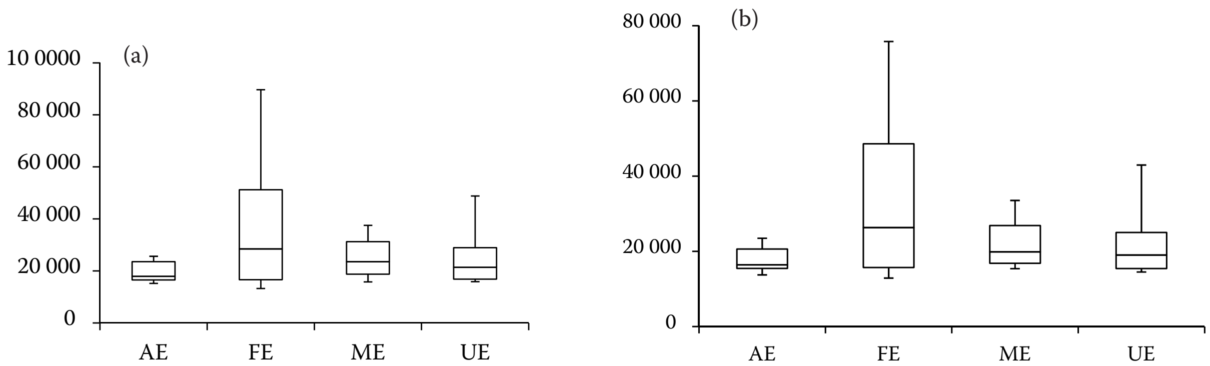

Figure 4. Total organic carbon (TOC, mg/kg) in the 0.5–1.0 mm fraction of dry-sieved macro-aggregates (a) and of water-resistant macro-aggregates (b)
AE – agro-ecosystem, FE – forest ecosystem, ME – meadow ecosystem, UE – urban ecosystem

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Table 3. Influence of total organic carbon (TOC) and labile forms of carbon in soil on the content of TOC and labile carbon (C_L) in dry-sieved macro-aggregates (DSA) and water-resistant (WSA) macro-aggregates

	DSA (mm)				WSA (mm)			
	TOC	C_L	CWEOC	HWEOC	TOC	C_L	CWEOC	HWEOC
TOC in aggregates								
>7	0.283	0.253	0.138	0.257	0.808**	0.761**	0.570**	0.771**
5–7	0.848**	0.763**	0.482**	0.752**	0.860**	0.765**	0.507**	0.769**
3–5	0.848**	0.762**	0.481**	0.752**	0.867**	0.780**	0.483**	0.718**
1–3	0.872**	0.800**	0.485**	0.739**	0.896**	0.821**	0.489**	0.713**
0.5–1.0	0.886**	0.811**	0.475**	0.713**	0.883**	0.807**	0.518**	0.756**
0.25–0.50	0.875**	0.856**	0.492**	0.696**	0.843**	0.841**	0.529**	0.785**
C_L in aggregates								
>7	0.826**	0.885**	0.614**	0.813**	0.647**	0.803**	0.559**	0.611**
5–7	0.899**	0.905**	0.452**	0.663**	0.858**	0.877**	0.576**	0.800**
3–5	0.894**	0.908**	0.431**	0.650**	0.863**	0.875**	0.578**	0.828**
1–3	0.797**	0.863**	0.584**	0.840**	0.907**	0.945**	0.522**	0.719**
0.5–1.0	0.907**	0.920**	0.500**	0.695**	0.892**	0.904**	0.531**	0.777**
0.25–0.50	0.863**	0.914**	0.545**	0.736**	0.693**	0.834**	0.491**	0.778**

CWEOC – cold water extractable organic carbon; HWEOC – hot water extractable organic carbon; $^{**}P < 0.01$

in the meadow ecosystem, i.e. in the natural ecosystem with a higher proportion of labile organic substances.

In the WSA and DSA size fractions 0.5–1.0 mm, the highest variability in the contents of TOC and C_L was recorded in the forest ecosystem > meadow ecosystem > urban ecosystem > agro-ecosystem. The higher were the inputs of organic substances into the soil, the greater was the variability in their incorporation into the soil aggregates.

The influence of the content of TOC and its labile forms on its contents in the DSA and WSA was different, and the contents of TOC and C_L in the aggregates were more significantly affected by the C_L content than by water soluble carbon. In the case of the WSA fractions, their carbon content was more affected in the 1–2 mm than in the 0.5–1.0 mm fraction.

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