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Parameters of labile organic carbon as the indicators of the stability of soil organic matter under different land use

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Abstract: The labile fractions of organic carbon (OC), which are a reflection of the properties of soil and its use, appear to be suitable parameters for their use as indicators. The aim of this study was to determine the reliable and relatively simple indicators for detecting the chemical and physical stabilizations of OC, which would respond sensitively to land use. The study includes forest ecosystem (FE) and agroecosystem (AE) with different tillage intensities (reduced tillage, RT and conventional tillage, CT) on real farms. Parameters of the labile C and N were tested. For a depth of < 0.1 m in the FE, the hot water extractable organic carbon (HWEOC) for chemical stabilization and labile nitrogen (N_L) for physical stabilization appear as the most suitable indicators. Higher values of HWEOC indicate the OC stabilization by decreasing decomposition, pH or by increasing carbonates, recalcitrant fractions, and higher values of N_L by OC incorporation into the silt fraction and larger macro-aggregates. In the AE with RT, these are the HWEOC for chemical stabilization and carbon pool index (CPI) or index of carbon lability (LI_C) for physical stabilization. Higher values of CPI and LI_C indicate the stabilization by the formation of size-optimal dry-sieved (DSA; 1–3 mm) and wet-sieved (WSA; 1–2 mm) soil aggregates. In the AE with CT, it was the N_L. Its higher values point to the stabilization through the carbonates, alkaline cations, size-fraction of > 0.01 mm and the formation of DSA (1–3 mm). For a depth of < 0.3 m in the AE, these are the C_L (for RT), higher value of which points to the stabilization by clay and alkaline cations, and HWEOC (for CT), higher value of which indicates the stabilization in the conditions of the soil acidification.

Keywords: agroecosystem; forest; indicators; labile carbon; stabilization; tillage

Assessment of soil organic carbon (SOC) stabilization mechanisms is key to understanding the carbon dynamics in the terrestrial ecosystems (Luo et al. 2024), as the dominance of factors depends on the ecosystem itself (Qu et al. 2019). Organic carbon in the soil can be stabilized chemically or biochemically by the

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formation of recalcitrant compounds (Song et al. 2013; Kilpeläinen et al. 2023), physically through the physical isolation in the soil aggregates (Cui et al. 2014; Zhong et al. 2017; Ji et al. 2024), or physicochemically through the sorption (Cui et al. 2014).

Labile fractions of the SOC are subject to stabilization. They are closely related to soil properties (Fu et al. 2023) and respond to changes in the soil parameters in a short time period, therefore they are sensitive indicators (Geraei et al. 2016; Duval et al. 2018; Chen et al. 2024b). The labile permanganate oxidizable carbon (C_L) (Bongiorno et al. 2019; Pulleman et al. 2021; Begum et al. 2022) and water extractable organic carbon (WEOC) (Zhao et al. 2008; Li et al. 2020) are the most using of the labile fractions in relation to soil quality and stability of organic substances. The determination of these labile fractions is simple, fast and inexpensive (Wang et al. 2017), which is consistent with the set requirements on the indicator (Dale & Beyeler 2001). The labile fractions of SOC show seasonal dynamics and are sensitive to environmental factors (Wang et al. 2022), including a land management system (Geraei et al. 2016; Liu et al. 2017).

The C_L is one of the labile fractions of organic carbon, which is often used as an indicator of changes in the soils of various ecosystems, at a various land use or soil management practice (Begum et al. 2022; Oliveira et al. 2022). Bongiorno et al. (2019) describe its high correlations with the chemical, physical, or biological parameters, and point to its use as a comprehensive soil quality indicator. Malou et al. (2023) consider the C_L to be an important indicator of SOC stability. The WEOC is often described as the most active labile fraction of organic carbon (Bu et al. 2011) and is used as an indicator of the soil quality, both in the agricultural soil, mainly in connection with the production ability (Bankó et al. 2021) and in the forest soil, mainly in a connection with natural disturbances (Wang et al. 2020). Together with labile nitrogen, there is a range of other parameters that respond sensitively to changes in the soil organic matter (SOM). Labile potentially mineralizable nitrogen (N_L) responds sensitively to tillage (Martínez et al. 2017), fertilization, and plays an important role in soil fertility (Hou et al. 2023). Thus, the labile fractions of SOM are considered to be the sensitive indicators in the management farming system in a short-time period (Sequeira et al. 2011). The carbon pool index (CPI) is valuable in the assessment of organic carbon turnover in the agroecosystem (Hu et al. 2023), and together with the carbon management index (CMI), also in the assessment of anthropogenic activities in a various land-use type (Huang et al. 2023). CPI is a quantitative parameter, which points to carbon sources in the ecosystem. CMI index is a combination of the quantity (CPI) and quality (LI_C) and determines the changes in soil organic carbon of agricultural and natural soils (Blair et al. 1995).

The aim of this study was to determine the reliable and relatively simple indicators, taking into account the chemical and physical stabilizations of organic matter, which would respond sensitively to interventions into the soil, especially to land use, soil tillage in the agroecosystem, especially in relation to a soil depth in this ecosystem, including the definition of the sensitive specific indicators and generally usable ones.

We hypothesized that the labile fractions of organic carbon are suitable for a determination of the changes in the organic carbon stability in a short time period. However, their suitability is strongly influenced by stabilization mechanisms in the ecosystem or by land use. Further, we hypothesise that the parameters of labile carbon are closely related to the soil properties, which reflect the land use and soil tillage; however, the depth of soil assessment is one of the most important factors that influence these properties.

MATERIAL AND METHODS

Characterization of the localities and variants included in the study. The studied areas were located in the lowland and hilly parts of western and eastern Slovakia. Neogene marine sediments are found in the geological subsoil of Slovak lowlands (Table 1).

Each area included the forest ecosystem and agroecosystem. The forest ecosystem represents control, as it is the broadleaf and mixed forests that are considered to be an original ecosystem in the temperate zone. Fernandes et al. (2022) also consider forest land as a suitable conservation reference for soil aggregation. The study included forests with a minimum lifespan of 200 years, with the original composition of trees, which means trees typical for the given soil type or locality. The agroecosystem included two tillage systems, each with three crop rotations. The arable land of the agroecosystem represented the real production conditions with two tillage systems, each with three repetitions in the form of various crop rotations. Reduced tillage (RT) as a non-inversion

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system included disking to a depth of 0.10–0.12 m (shallow tillage) and conventional tillage (CT) as an inversion system included moldboard deep ploughing to a depth of 0.25–0.30 m (deep tillage). The average values of the basic parameters of the included soils are summarized in Table 2.

Soil samples were taken separately at two depths (0.0–0.1 and 0.0– 0.3 m) at each sampling point. This means 378 sampling sites (7 soil types × 3 localities × 3 land use × 3 repetitions × 2 depths).

Soil samples and analytical methods used. The soil samples were dried at a room temperature 25 ± 2 °C. For the determination of the physical properties, the samples were used in their non-disrupted state, and for the determination of the chemical properties, they were sieved (2 and 0.25 mm). To a determination of the fractions of soil aggregates, the soil samples were divided by sieves (Sarkar & Haldar 2005). The fractions of dry-sieved macro-aggregates (DSA) were as followed: > 7; 5–7; 3–5; 1–3; 0.5–1; 0.25–0.5 mm and wet-sieved (WSA): > 5; 3–5; 2–3; 1–2; 0.5–1; 0.25–0.5 mm. 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 to the pipette method (van Reeuwijk 2002). The total organic carbon (TOC) (Skjemstad & Baldock 2007) and labile permanganate oxidizable carbon (C_L) (Loginov et al. 1987) were determined by wet combustion by $K_2Cr_2O_7$ and $KMnO_4$ oxidations. Both were used for the calculation of the next parameters: lability of carbon (L_C); index of carbon lability (LI_C); carbon pool index (CPI); carbon management index (CMI); according to Blair et al. (1995) as followed:

$$C_{NL}\ \text{(non-labile carbon)} = TOC - C_L$$

$$L_C = C_L/C_{NL}$$

$$LI_C = L_C\ \text{in variant}/L_C\ \text{in control}$$

$$CPI = TOC\ \text{in variant}/TOC\ \text{in control}$$

$$CMI = LI_C \times CPI \times 100$$

Table 1. Characterization of the localities

Geographical location						
Danubian Lowland		Trnava Hills	Žitava Hills	Eastern Slovak Lowland		
Substrate*						
Carbonate gravels, sands, clays		carbonate loess; along some tectonic faults, Neogene marine sediments have reached the surface		non-carbonate clay sediments		
Soil type***						
Mollic Fluvisol	Eutric Regosol	Haplic Chernozem	Haplic Luvisol	Eutric Gleysol	Stagnic Planosol	Eutric Fluvisol
Locality						
Nové Zámky (Nové Zámky, Komoča, Šurany)	Šaľa (Šaľa, Močenok, Horná Králová)	Piešťany (Piešťany, Trebatice, Krakovany)	Vráble (Vráble, Nová Ves n./Žitavou, Horný Ohaj),	Michalovce (Hažín, Petrikovce, Lúčky)	Sobrance (Blatné Revištia, Blatná Polianka, Bežovce)	Trebišov (Milhostov, Kráľovský Chlmec, Streda n./Bodrogom)
Climatic region**						
Warm				moderately warm		
Average annual temperatures (°C)**						
9.4–10.4				9–9.1		
Average rainfall per year (mm)**						
566–611				564–593		
Altitude (m a.s.l.)						
110–229				98–850**		

*Bedrna and Jenčo (2016); **Korec et al. (1997); ***WRB (2015)

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Table 2. The average values of basic parameters of the monitored soils (< 0.1 m)

Soil type	TOC	C_L	HWEOC	N_L	pH_{KCl}	Clay
	(g/kg)					(%)
Forest ecosystem						
EF	24.32 ± 3.27	2.36 ± 0.21	1.33 ± 0.29	0.15 ± 0.02	6.87 ± 0.17	23.59 ± 0.24
MF	43.24 ± 1.95	3.33 ± 1.64	1.53 ± 0.39	0.13 ± 0.04	6.75 ± 0.83	29.83 ± 2.47
HC	23.95 ± 1.25	2.29 ± 1.18	1.69 ± 0.34	0.12 ± 0,04	6.31 ± 1.03	20.70 ± 0.71
HL	26.77 ± 2.47	4.89 ± 1.12	1.96 ± 0.16	0.15 ± 0.01	5.43 ± 0.26	22.09 ± 0.56
ER	22.46 ± 1.59	4.32 ± 1.54	1.89 ± 0.33	0.17 ± 0.03	5.60 ± 0.18	16.39 ± 0.30
EG	42.18 ± 4.15	5.42 ± 1.77	1.78 ± 0.13	0.16 ± 0.01	5.65 ± 0.30	37.70 ± 1.28
SP	26.63 ± 1.07	3.04 ± 1.46	1.41 ± 0.26	0.13 ± 0.03	5.06 ± 0.41	24.03 ± 0.81
Agroecosystem – reduced tillage						
EF	23.20 ± 2.56	2.06 ± 0.28	1.19 ± 0.09	0.16 ± 0.03	6.89 ± 0.24	23.39 ± 1.45
MF	26.96 ± 2.24	2.45 ± 0.37	1.40 ± 0.15	0.12 ± 0.03	6.80 ± 0.05	29.88 ± 2.41
HC	21.47 ± 1.79	2.26 ± 0.51	1.27 ± 0.20	0.11 ± 0.03	6.68 ± 0.48	21.57 ± 2.06
HL	17.59 ± 2.47	1.76 ± 0.62	1.17 ± 0.13	0.13 ± 0.03	6.15 ± 0.38	20.69 ± 2.50
ER	11.93 ± 1.60	1.88 ± 0.65	1.16 ± 0.18	0.14 ± 0.02	5.97 ± 0.16	15.05 ± 0.57
EG	23.40 ± 2.17	2.01 ± 0.24	1.24 ± 0.16	0.12 ± 0.01	5.58 ± 0.54	35.78 ± 1.86
SP	14.43 ± 2.24	1.27 ± 0.16	0.96 ± 0.08	0.10 ± 0.02	5.05 ± 0.59	22.75 ± 2.67
Agroecosystem – conventional tillage						
EF	19.45 ± 1.89	1.48 ± 0.30	1.02 ± 0.10	0.12 ± 0.03	6.60 ± 0.57	25.18 ± 4.05
MF	38.01 ± 2.35	3.50 ± 1.17	1.42 ± 0.32	0.15 ± 0.04	6.87 ± 0.37	34.74 ± 2.25
HC	14.70 ± 1.38	1.67 ± 0.13	1.18 ± 0.06	0.11 ± 0.02	6.34 ± 0.11	25.63 ± 1.17
HL	16.15 ± 1.02	1.57 ± 0.19	1.13 ± 0.13	0.11 ± 0.02	6.39 ± 0.42	21.22 ± 1.53
ER	12.07 ± 1.48	1.90 ± 0.42	1.10 ± 0.13	0.11 ± 0.02	5.96 ± 0.18	16.13 ± 1.07
EG	21.17 ± 2.73	2.69 ± 1.14	1.14 ± 0.22	0.10 ± 0.02	5.75 ± 0.59	31.15 ± 2.20
SP	19.10 ± 2.74	1.86 ± 0.46	0.85 ± 0.30	0.11 ± 0.01	5.49 ± 0.44	25.35 ± 1.57

EF – Eutric Fluvisol; MF – Mollic Fluvisol; HC – Haplic Chernozem; HL – Haplic Luvisol; ER – Eutric Regosol; EG – Eutric Gleysol; SP – Stagnic Planosol; TOC – total organic carbon; C_L – labile carbon oxidizable with $KMnO_4$; HWEOC – hot water extractable organic carbon; N_L – labile nitrogen

Cold (CWEOC) and hot (HWEOC) water extractable organic carbons were determined by distilled water extraction (20 °C and 80 °C) according to the method Ghani et al. (2003) with the final determination of organic carbon by wet combustion (Skjemstad & Baldock 2007). Potentially mineralizable soil nitrogen (N_L) was determined by acid $KMnO_4$ extraction method (Standford & Smith 1978). Soil pH was potentiometrically measured (van Reeuwijk 2002) in a supernatant suspension of a 1/2.5 soil/liquid mixture. The liquid is 1 mol/dm^3 KCl (pH/KCl). Electrical conductivity was measured in aqueous suspension in a soil/water ratio of 1/2.5 (Rhoades 1982). Carbonates were determined by volumetric method (using a simple calcimeter), based on the CO_2 evolving after reaction with HCl (diluted with water in a 1/3 ratio) (Sarkar & Haldar 2005). The hydrolytic acidity (H_a) in calcium acetate at pH 8.2 and sum of exchangeable cations (S) with 0.1 mol/dm^3 of hydrochloric acid were determined according to Kappen's method (Jaremko & Kalembasa 2014), and then the total sorption capacity (T) and base saturation (V) were calculated as followed:

$$T = S + Ha; V = S/T \times 100$$

Statistical analysis used in the study. The obtained data were analysed using Statgraphic Plus 5.1 and Centurion 17 statistical software. Firstly, the normality and homogeneity of variances were tested, and then analysis of variance was applied. ANOVA model was used for individual treatment comparisons

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at $P < 0.05$, with separation based on Fisher's least significant difference (LSD) procedure (three files with $n = 378$, 186, and 126) applied to test for differences between the depths (0.0–0.1 m and 0.0–0.3 m) for TOC, C_L, CWEOC, HWEOC. The results are presented in the boxplots as mean, 2Q box, 3Q box, error bars. Correlation analysis was used to determine the relationships between the parameters of organic carbon or nitrogen and chemical or physical properties of the soils. Significant Pearson correlation coefficients were tested at $P < 0.05$, $P < 0.01$, and $P < 0.001$.

RESULTS AND DISCUSSION

General indicators concerning to chemical properties of the soil. Studied labile fractions of organic carbon and nitrogen and their parameters appear to be important indicators not only of the SOM stability, but also of several soil properties. According to Bongiorno et al. (2019) and Ramírez et al. (2020), they are also a reflection of human activity. Our results point to an increase in the proportions of labile forms of organic carbon at a lower pH and at a higher H_a, while the correlations with H_a were much stronger (Figure 1). The main reason for this decrease in soil pH is CO_2 that is released from the decomposing organic sources (Xiao et al. 2018). On the other hand, the activity of soil organisms decreases with the decrease in pH (Qiu et al. 2023a). Our values point to an accumulation of labile forms after decreasing microbial activity at a lower soil pH. Liang et al. (2024) pointed to limiting values of soil pH below 3.5 and above 7.5, when in combination with clay content significant changes occur both in the microbial activities and sorption. The evidence of which, the correlations of the C_L, L_C, CWEOC and HWEOC with pH/KCl and H_a are (Figure 1). In relation to microbial activity the active soil pH is mainly presented. A higher amount of WEOC was at a higher soil acidity, as well. Kupka and Gruba (2022) also presented that the sorption of dissolved organic carbon increases with an increase in soil acidity or with a decrease in active pH. HWEOC, as the most sensitive parameter of the changes in SOC, was presented also by Li et al. (2020). Overall, the pH significantly influences the dynamics of organic carbon in the soil, which is confirmed by the results of active pH (Hao & Dong 2023). Thus, higher contents of the labile forms of carbon can be the result of accumulated labile inputs of carbon.

General indicators concerning to physical properties of the soil. The L_C values were also significantly influenced by the fractions of the clay ($r = -0.349$, $P < 0.05$) and silt ($r = 0.441$, $P < 0.01$). Clay contributes not only to the stabilization of organic substances in the soil (Arthur et al. 2023), but a significant amount of the organic carbon is also a part of the silt and clay fractions (Zhao et al. 2006; Chen et al. 2024a). On the contrary, in the case of soil aggregates, the L_C was the alone monitored parameter that did not show any correlations neither with the DSA nor with WSA. Differences in the carbon lability were not observed due to its different lability in the various fractions of aggregates, in which it is incorporated (Yu et al. 2022). The N_L is a parameter that was importantly manifested in relation to soil aggregates, but it did not manifest in relation to the chemical properties or soil texture. Fungi are more important in aggregate formation than bacteria (Husain et al. 2024), but they are also an important mediator of nitrogen, through which they support OC decomposition (Bai et al. 2024). N_L was in a narrow dependence with the size-optimal fractions of the DSA (1–5 mm), but not with the WSA (0.5–2 mm), and simultaneously, the opposite correlation of C_L/N_L ratio was recorded (Figure 2). In the case of DSA, the nitrogen is a part of fresh inputs, but in the case of WSA, the C_L/N_L ratio decreases in the stabilization process of organic substances. Nitrogen even could have a destabilizing

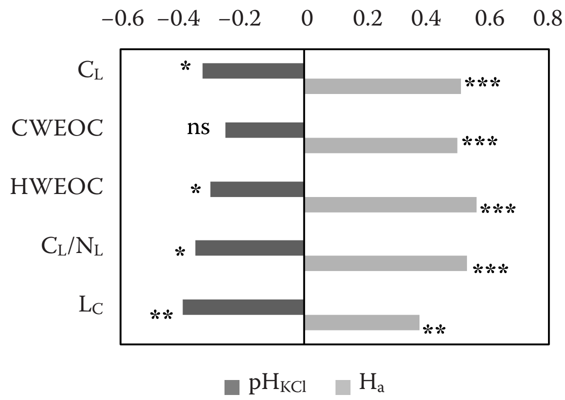

Figure 1. Correlations between the parameters of carbon and nitrogen and the pH_{KCl} and hydrolytic acidity (H_a); general for both ecosystems

C_L – labile carbon oxidizable with $KMnO_4$; CWEOC – cold water extractable organic carbon; HWEOC – hot water extractable organic carbon; C_L/N_L – ratio of labile carbon and labile nitrogen; L_C – lability of carbon; *,**,***$P < 0.05$, 0.01 and 0.001; ns – not significant

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effect on them (Xiao et al. 2021; Zhao et al. 2024). The above points to the fact that the sensitivity of the mentioned parameters depends also on the chemical and physical stabilization mechanisms of organic carbon itself. In relation to nitrogen it is also an important the size of fresh residues, because it influences the diversity of microbial community on them (Ji et al. 2024), which is in a close relation to labile nitrogen, which is bound in these cells. The presence of fresh organic components in the DSA is also indicated by a positive correlation of N_L with DSA, while a negative correlation with WSA indicates already its decomposition or stabilization. The proportion of fresh inputs decreases with a decrease in the aggregate size, which is also confirmed by a positive correlation of organic nitrogen content with the aggregate size (Liu et al. 2023b). However, the most agronomically valuable WSA were in negative correlations with the labile fractions of organic carbon (C_L, CWEOC, HWEOC), including C_L/N_L ratio, indicating that higher amounts of these aggregates are at less proportions of labile sources. Moreover, the increased inputs of labile carbon offset the negative effects of applied nitrogen on the soil aggregates (Zhao et al. 2024). Conversely, the same organic carbon fractions, including the C_L/N_L ratio, were in positive correlations with the largest WSA fraction, indicating the importance of physical stabilization of the labile forms of organic matter. In our climate zone, the formation of soil aggregates is dominated by the „bottom-up "model (Jiang et al. 2023); at first, the small aggregates are formed, and then the larger ones from them are built. Simultaneously, it is true that the free micro-aggregates are the fragments of disintegrated macro-aggregates (Artemyeva et al. 2022). According to these theories, the disintegration of larger aggregates leads to releasing of labile organic substances, which subjects to decomposition, that means that lower values of C_L/N_L ratio and organic carbon fractions indicate higher amounts of smaller aggregates, and thus they can be important indicators of the carbon stabilization. While all mentioned correlations were negative for WSA, in the case of DSA, they were positive ones. DSA are important in the assessment of soil management systems (Guo et al. 2021). Tillage leads to a more significant disturbance of the larger DSA (Xiao et al. 2019); thereby, the proportion of smaller DSA increases. Since the labile components dominate in the larger aggregates, the results are the positive correlations between the labile carbon fractions and the proportion of smaller aggregates. The WSA are water-resistant aggregates, and the organic carbon is more stabilized in them. However, the DSA are aggregates in which the organic carbon is initially accumulated (Six et al. 2002), and then it is consequently decided on its stabilization mechanisms in the WSA.

If the mentioned fractions are assessed at the ecosystem level or soil management, the influence of certain factors is filtered out, thereby the accuracy of the assessment increases.

Specific indicators for the forest ecosystem. In the case of a forest ecosystem, the statistically significant correlations between the HWEOC and pH/KCl (r = −0.574, P < 0.05) or H_a (r = 0.547, P < 0.05) were recorded. At a higher extraction temperature (80 °C) and at the same time protein denaturation, the microbial cells that were originally bound to the sorption complex are extracted (Sparling et al. 1998), whereas in the case of a forest ecosystem, the reason may be also an increased fungi proportion, which are dominant organisms in the acidic conditions (Zhou et al. 2021). Moreover, the HWEOC appears to be the most sensitive labile fraction that is responding to changes in the SOC (Li et al. 2020; Wang et al. 2020).

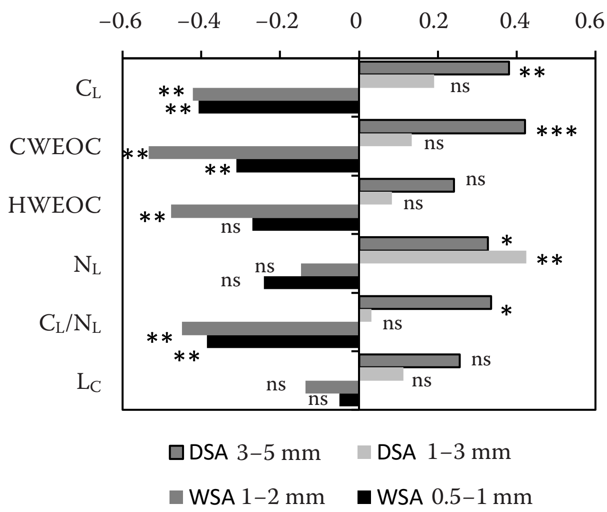

Figure 2. Correlations between the parameters of carbon and nitrogen and the fractional composition of dry-sieved (DSA) and wet-sieved (WSA) soil aggregates; general for both ecosystems

C_L – labile carbon oxidizable with $KMnO_4$; CWEOC – cold water extractable organic carbon; HWEOC – hot water extractable organic carbon; N_L – labile nitrogen; C_L/N_L – ratio of labile carbon and labile nitrogen; L_C – lability of carbon; *,**,***P < 0.05, 0.01 and 0.001; ns – not significant

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In the forest ecosystem, the significant differences between the labile fractions of organic carbon were recorded, even in the case of soil depths (Figure 3), contrary to the agroecosystem, in which the differences are erased by tillage because the depth of the tillage on all fields was nearly the same in the past.

The higher TOC contents in the soils of the forest ecosystem were on average in a depth of 0.0–0.1 m, while it is not only the result of a natural ecosystem specificity but also the response of the forest itself to an organic carbon distribution in the soil profile of a forest soil (Song et al. 2017). C_L values were significantly higher at a depth of 0.0–0.1 m, however the differences in the contents of CWEOC and HWEOC were less pronounced. The restoration of WEOC in the upper layer is bound to rebuilding the organic layer (Wang et al. 2020), which is important mainly in the forest ecosystem. The distribution of WEOC in the soil profile is one of the most intensive, and simultaneously, its fractions respond more sensitively to rapidly changing conditions. The WEOC is one of the most mobile and the most accessible fractions for microorganisms (Fissore et al. 2017). In the case of a forest ecosystem, thus it will be the most suitable indicator relating to a depth of 0.0–0.1 m.

From the mentioned labile fractions of the organic carbon, it was again the HWEOC, which was in the forest ecosystem in a depth of 0.0–0.1 m in negative correlations with the content of carbonates (r = –0.795, P < 0.05) and pH/KCl (r = –0.814, P < 0.05). The structure of microbial community is determined by pH, which was also reflected in a correlation with HWEOC (Guigue et al. 2015). However, the HWEOC has not impact on the changes in pH and Ca^{2+} (Zhao et al. 2024). L_C parameter was in a depth of 0.0–0.1 m in a negative correlation with all chemical properties, but in a depth of 0.0–0.3 m, it was the only parameter of all, which pronounced in this depth. This indicates a higher stability of organic carbon at a higher pH/KCl (r = –0.641, P < 0.05), content of alkaline cations (S) (r = –0.661, P < 0.05), total sorption capacity (T) (r = –0.632, P < 0.05). However, the mechanism of WEOC stabilization by alkaline cations in saline-alkaline soil is different, because in these conditions, the influence of $CaCO_3$ is suppressed by a high Na^+ concentration (Wu et al. 2021).

In relation to physical properties in the forest ecosystem to a depth of 0.1 m (Figure 4), the N_L parameter appears to be the most sensitive one.

In the case of soil texture, a negative correlation of N_L with the sand fraction and a positive correlation with the silt fraction were recorded at a depth of 0.0 – 0.1 m. Almost 80% of all organisms are found at a depth up to 0.1 m (Qiu et al. 2023b), of fungi up to 0.2 m (Xiao et al. 2024). These are dominant in the forest ecosystem in the upper part of the soil profile, and they mediate the nitrogen to photosynthesizing plants. In the case of DSA, the

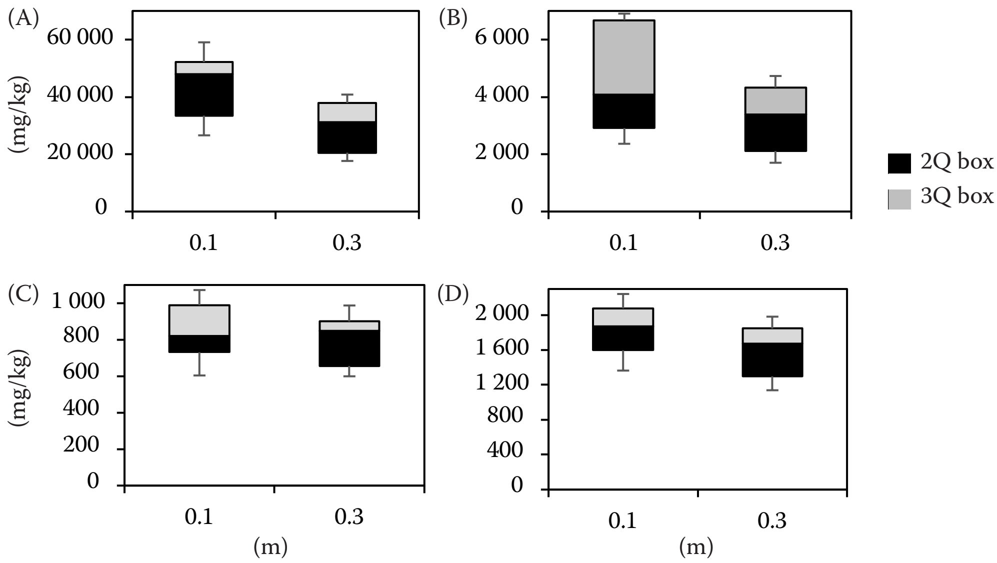

Figure 3. Differences in: total organic carbon (TOC) (A), labile carbon (C_L) oxidizable with $KMnO_4$ (B), cold water extractable organic carbon (CWEOC) (C), hot water extractable organic carbon (HWEOC) (D), with dependence to soil depth in the forest ecosystem
P < 0.05

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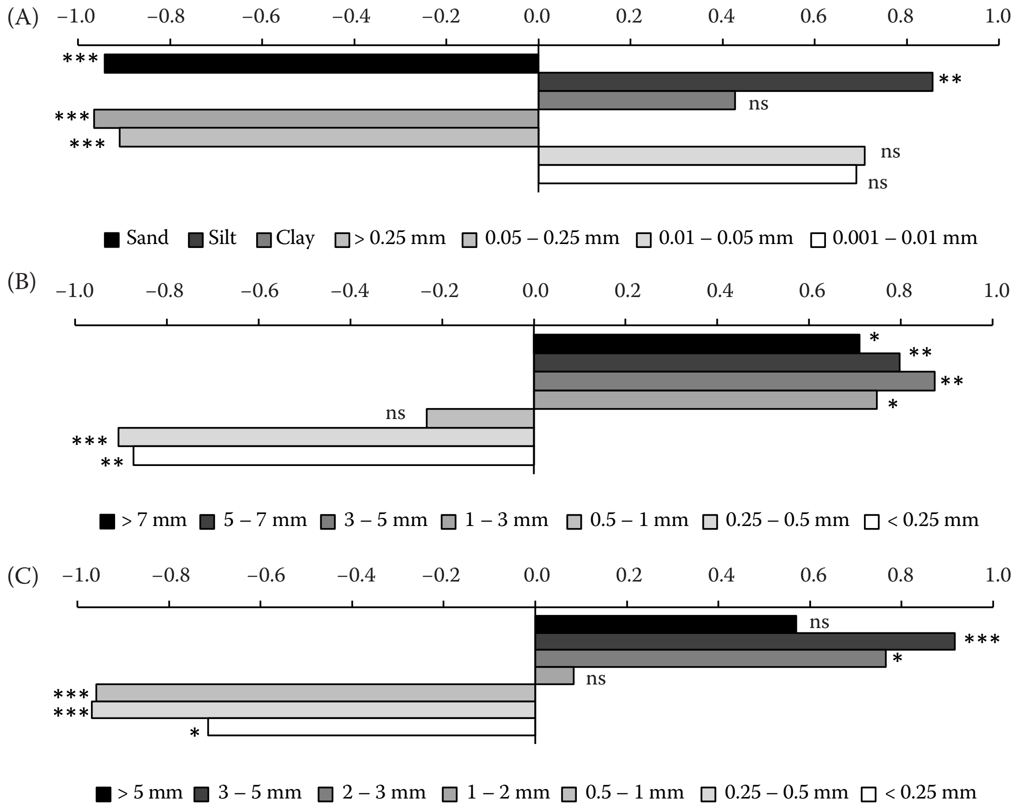

Figure 4. Correlations between the labile nitrogen (N_L) and: particle size distribution (A), fractions of dry-sieved aggregates (DSA) (B), fractions of wet-sieved aggregates (WSA) (C), in the forest ecosystem at a depth of 0.0–0.1 m

N_L at a depth of 0.0–0.1 m was in a correlation with almost all fractions. While, in the case of larger fractions in a positive correlation, in the case of smaller fractions in a negative correlation that again indicates the importance of fungi, mainly their contribution to the mechanical formation of soil aggregates through mycelia. Fungi are important mainly in the larger aggregates immediately after the ploughing (Dal Ferro et al. 2023). In the case of smaller aggregates, the enzyme activity of fungi is higher, and the dominant mechanism of stabilization is through secretion-based bonding or hydrophobicity modulation (Liu et al. 2023a). In the case of larger aggregates, there are the fungi mycelia, and the physical mechanism of stabilization is dominant. However, in both the smaller and larger aggregates, the labile nitrogen contents increase. Similar correlations of N_L as in the WSA were found in the in DSA.

Specific indicators for the agroecosystem. In the agroecosystem, the carbon parameters were extended by LI_C, CPI, and CMI, and just in the case of these parameters, the correlations with the carbonate contents, pH/KCl, and the parameters of the sorption complex were recorded. In the case of the agroecosystem, the reduced tillage was manifested differently in the depth of the soil sampling (Table 3). In the case of the sorption complex at a depth of 0.0–0.1 m, there were mainly correlations with the C_L/N_L ratio, L_C, and CPI, thus with the secondary parameters, however in the case of a depth of 0.0–0.3 m with primary ones, so with the specific labile carbon fractions (C_L, CWEOC, HWEOC).

While at a depth of 0.0–0.1 m, the stability of the soil ecosystem was manifested in the form of the above correlations, at a depth of 0.0 – 0.3 m, it was an especially manifestation of the stability of organic carbon in a specific time and place. In deeper parts of the soil profile, the properties of soil have a strong influence on the carbon stock (Lăcătușu et al. 2024), which means that the mechanisms of its stabilization are closely related to soil genesis, and they can be less influenced by land use. At a depth of 0.0–0.1 m, a higher value of H_a and a lower pH/KCl indicated higher carbon sources (CPI). However, at the higher proportions of carbonates and alkaline cations, the stability of organic carbon (LI_C) in the agroecosystem was higher. In the reduced tillage system, most of the organic inputs are concentrated in a depth of 0.0–0.1 m. In the decomposition process of organic substances, the acidic components are produced (Buresova et al. 2021), therefore, if all inputs are accumulated only to this depth and at their simultaneous decomposition, the CPI value increases. The intensity of the transformation processes increases when the environmental conditions are optimized. In this case, a sufficient proportion of carbonates and alkaline cations participated not only in the neutralization of newly formed acidic components but also contributed to an increase in microbial activity and, thus, the labile sources of organic carbon, which reflected in a higher LI_C value. The stabilizing effect of carbonates is well known (Martí-Roura et al. 2019). At lower values of pH, the minerals become also more soluble, and thus, the nutrients become more accessible (Ferrarezi et al. 2022). At a depth of 0.0–0.3 m, under the mentioned conditions, there is a higher proportion of the labile fractions of carbon (C_L, CWEOC, HWEOC), the sources of which in this depth are the roots, root exudates, and microbial biomass of the rhizosphere zone, and simultaneously the organic components leached from the surface parts of the soil profile (Bellmore et al. 2015). The next changes in the OC are driven by soil properties (Xu et al. 2024) in a relevant part of the soil profile. Overall, thus the carbon contents are not lower, but only distributed to a greater depth.

With regard to soil structure, a more significant variability in the parameters was at a depth of 0.0 to 0.1 m, which is related to the aggregation process itself. Various tillage systems influence differently the formation of soil aggregates (de Souza et al. 2024), whereas the most significant differences in the influence of tillage are at a depth of 0.00–0.05 m (Büchi et al. 2022). At a depth of 0.0–0.1 m (Table 4), the correlations on the level of overall ecosystem stability were again dominated, however, at a depth of 0.0–0.3 m those that point to the stability of organic carbon at a specific time and place.

However, the positive correlations of the lability and labile fractions with larger (> 5 mm) and negative with smaller (< 1 mm) fractions of DSA were recorded in both depths. In the larger aggregates, there is more labile organic carbon, which also subjects to greater changes (Lee et al. 2009), which are related not only to the availability of the substrate for decomposers in connection to the size of soil pores but also to the mechanism of aggregation itself. The highest microbial activity is in the pores of the range of 40–90 μm and these pores are associated with a higher mineralization of the new organic inputs, while the pores of < 40 μm size are associated with carbon stabilization (Quigley et al. 2018). Larger pores mean a higher intensity of the mineralization and lower stability of the aggregates (Ma et al. 2021), the result of which is a lower carbon stabilization in the larger aggregates.

The relationships between the parameters of the lability of organic matter in the soil and the fractional composition of WSA under reduced tillage were manifested only at a depth of 0.0–0.1 m (Table 5). The

Table 3. Correlations between the parameters of organic carbon and chemical properties in the agroecosystem with a reduced tillage

	H_a	S	T	V	CO_3^{2-}	pH_{KCl}
0.0–0.1 m						
LI_C	ns	0.446*	0.473*	ns	0.581**	ns
CPI	0.796***	ns	ns	–0.564**	ns	–0.577**
CMI	ns	ns	ns	ns	ns	ns
0.0–0.3 m						
C_L	ns	0.453*	0.449*	0.445*	ns	0.442*
CWEOC	ns	0.479*	0.483*	ns	ns	0.463*
HWEOC	ns	0.592**	0.607**	ns	0.553**	ns

LI_C – index of carbon lability; CPI – carbon pool index; CMI – carbon management index; C_L – labile carbon oxidizable with $KMnO_4$; CWEOC – cold water extractable organic carbon; HWEOC – hot water extractable organic carbon; H_a – hydrolytic acidity; S – sum of exchangeable cations; T – total sorption capacity; V – base saturation; CO_3^{2-} – contents of carbonates; *,**,***P < 0.05, 0.01 and 0.001; ns – not significant

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Table 4. Correlations between the parameters of organic carbon and fractional composition of dry-sieved soil aggregates (DSA) in the agroecosystem with a reduced tillage

	> 7	5–7	3–5	1–3	0.5–1	0.25–0.5	< 0.25
				(mm)			
0.0–0.1 m							
LI_C	0.440*	0.659***	ns	–0.427*	–0.562**	–0.475*	ns
CPI	ns	ns	ns	ns	ns	ns	–0.447*
CMI	0.491*	0.620**	ns	ns	–0.516*	–0.446*	–0.500*
0.0–0.3 m							
C_L	ns	0.484*	ns	ns	–0.461*	–0.622**	–0.565**
CWEOC	ns	ns	ns	ns	ns	ns	ns
HWEOC	ns	0.465*	ns	ns	ns	ns	ns

LI_C – index of carbon lability; CPI – carbon pool index; CMI – carbon management index; C_L – labile carbon oxidizable with $KMnO_4$; CWEOC – cold water extractable organic carbon; HWEOC – hot water extractable organic carbon; *,**,****P* < 0.05, 0.01 and 0.001; ns – not significant

important finding is that it is a correlation of agronomically the most size-valuable aggregates (1–2 mm), which indicates that their higher proportion is at a higher proportion of the stabile organic sources and simultaneously at a lower intensity of changes. Cheng et al. (2023) presented that the SOC content is the highest in these aggregates (0.25–2 mm), but the amount of organic carbon in the aggregates does not correspond to its higher stability. Overall, the stability of soil aggregates varies depending on the organic matter content, clay content and pH (Kodešová et al. 2009; Pavlů et al. 2022), but their total stability is also the result of a greater water repellence (Tomashefski & Slater 2023).

On the contrary, in the case of conventional tillage, the correlations were manifested only at a depth of 0.0–0.3 m (Table 5). They were the correlations of the same parameters (LI_C, CPI, CMI) with agronomically the most valuable WSA (1–3 mm), with a shift of the WSA fraction of the size 2–3 mm, which points to a more favourable influence of the conventional tillage. The formation of macro-aggregates is associated mainly with labile carbon sources (Zhang et al. 2017), which have a greater proportion in deeper parts of the soil profile in the conventional tillage due to the incorporation of post-harvest residues or organic fertilizers. The accumulation of organic matter in the larger aggregates is associated with carbon sequestration (Xiao et al. 2021a, b).

Table 5. Correlations between the parameters of organic carbon and fractional composition of the wet-sieved soil aggregates (WSA) in the agroecosystem with a different tillage

	> 5	3–5	2–3	1–2	0.5–1	0.25–0.5	< 0.25
				(mm)			
Reduced tillage (0.0–0.1 m)							
LI_C	0.529**	ns	ns	–0.513*	ns	ns	ns
CPI	ns	0.432*	ns	ns	ns	ns	ns
CMI	0.603**	0.445*	ns	–0.585**	–0.499*	ns	ns
Conventional tillage (0.0–0.3 m)							
LI_C	ns	ns	–0.462*	–0.502*	ns	ns	ns
CPI	ns	ns	–0.561**	–0.728***	ns	ns	ns
CMI	ns	ns	–0.712***	–0.797***	ns	ns	0.447*

LI_C – index of carbon lability; CPI – carbon pool index; CMI – carbon management index; *,**,****P* < 0.05, 0.01 and 0.001; ns – not significant

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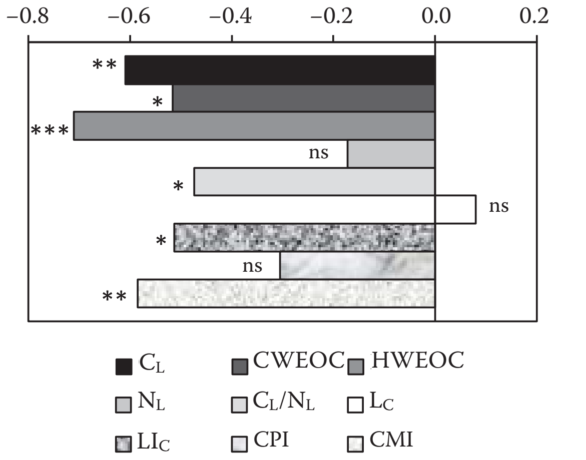

Figure 5. Correlations between the parameters of organic carbon and the wet-sieved aggregates (WSA) of the size 1–2 mm in the agroecosystem with reduced tillage at a depth of 0.0–0.1 m

C_L – labile carbon oxidizable with $KMnO_4$; CWEOC – cold water extractable organic carbon; HWEOC – hot water extractable organic carbon; N_L – labile nitrogen; C_L/N_L – ratio of labile carbon and labile nitrogen; L_C – lability of carbon; LI_C – index of carbon lability; CPI – carbon pool index; CMI – carbon management index; *,**,****P* < 0.05, 0.01 and 0.001; ns – not significant

Overall, a higher content of WSA of the size 1–2 mm was at a lower content of all labile carbon fractions (C_L, CWEOC, HWEOC) and a narrower C_L/N_L ratio (Figure 5).

The influence of tillage on the proportion of agronomically the most valuable WSA fractions was more pronounced in the case of reduced tillage at a depth of 0.0–0.1 m, while in the case of conventional one at a depth of 0.0–0.3 m, so in both cases to a maximum depth of contemporary tillage. The tillage system influences the differences in aggregate fractions and is in a close relation to bacterial community (Wang et al. 2019), which is reflected in the correlations with the various carbon fractions.

Since, in relation to the other observed parameters, relatively the same correlations were found in both tillage systems, and they were significantly manifested mainly in the agronomically the most valuable WSA fractions, these parameters (LI_C, CPI, CMI) can be considered key indicators of the impact of tillage on both the stability of organic carbon and soil aggregates themselves, since up to 90% of organic carbon is stabilized in aggregates (Kan et al. 2020).

In the case of soil texture, the concordance at both depths in the correlations was observed (Table 6). A positive correlation between the C_L and clay fraction and a negative correlation between the CPI and sand fraction point to the higher carbon sources in the soils with a higher clay proportion, in spite of higher content of labile carbon. The presence of clay, Ca^{2+}, and organic carbon favour organo-mineral interactions (Laranjeira et al. 2024), especially through the cation bridges. However, the influence of soil texture on organic carbon stabilization can be not only direct, through the mechanisms of physico-chemical or physical stabilization, but also indirect, through the influencing of microbial activity (Liao et al. 2024).

In comparison to the forest ecosystem, in the agroecosystem, the values of TOC and its labile fractions in both depths are relatively equal (Figure 6). The largest differences are in TOC, which includes all organic carbon in the soil, including the recent inputs, so its values are slightly higher at a depth of 0.0–0.1 m. However, over time, the decomposition partly occurs, mainly of labile carbon and simultaneously, a part of organic carbon is distributed into deeper parts of the soil profile by which the differences of C_L

Table 6. Correlations between the parameters of organic carbon and particle size distribution in the agroecosystem with a reduced tillage

	Sand	Silt	Clay	> 0.25	0.05–0.25	0.01–0.05	0.001–0.01
				(mm)			
0.0–0.1 m							
C_L	ns	ns	0.461*	ns	ns	ns	ns
CPI	–0.580**	ns	0.483*	ns	–0.586**	ns	ns
0.0–0.3 m							
C_L	ns	ns	0.463*	–0.428*	ns	ns	ns
CPI	–0.493*	ns	0.409*	–0.475*	–0.473*	0.534*	ns

C_L – labile carbon oxidizable with $KMnO_4$; CPI – carbon pool index; *,***P* < 0.05, 0.01; ns – not significant

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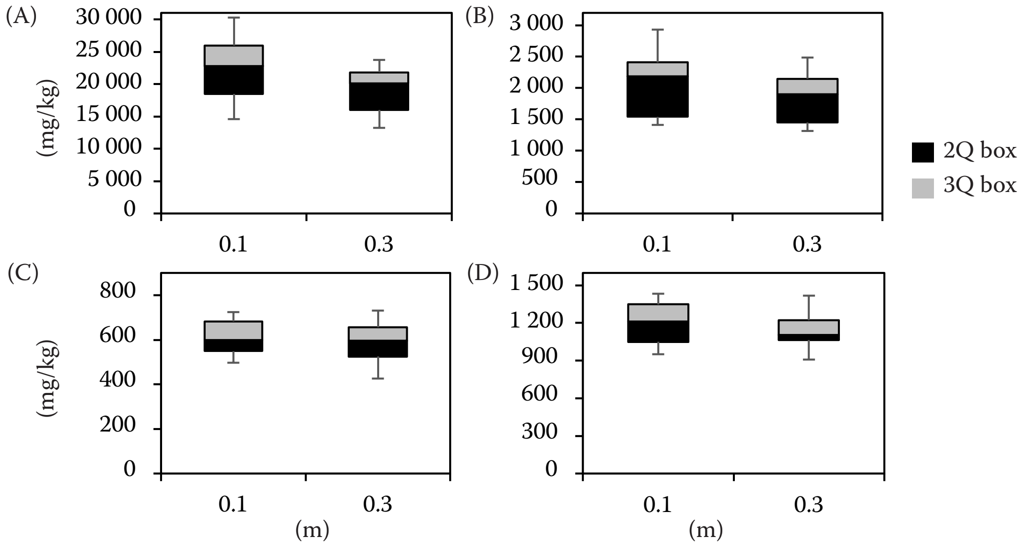

Figure 6. Differences in: total organic carbon (TOC) (A), labile carbon (C_L) oxidizable with $KMnO_4$ (B), cold water extractable organic carbon (CWEOC) (C), hot water extractable organic carbon (HWEOC) (D), with dependence on a soil depth in the agroecosystem with reduced tillage
$P < 0.05$

contents between the depths reduce. The WEOC/TOC ratio increases with the depth of the soil profile (Corvasce et al. 2006). The contents of CWEOC and HWEOC at both depths are almost equal, as they are by water-extractable forms that leached into deeper parts of the soil profile continuously and faster. The vertical distribution of OC in the soil profile is influenced mainly by environmental factors (Zhang et al. 2024). Brevilieri et al. (2024) did not find substantial differences in the carbon stock between the chiselling and no-till systems, and so recommend the assessment of organic carbon stock not only up to 0.3, but up to 1 m. Thus, the evaluation of the tillage effect on the SOC and its fractions is appropriate

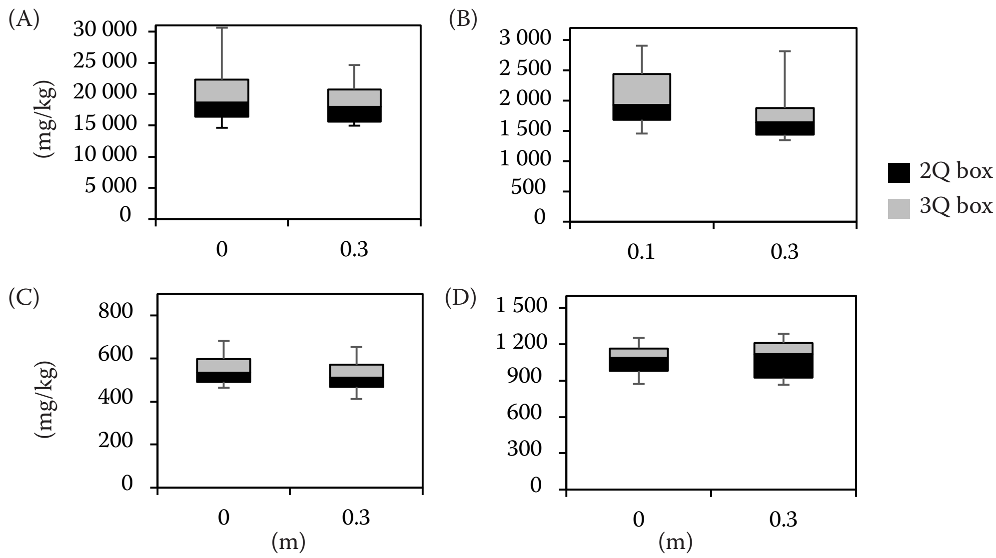

Figure 7. Differences in: total organic carbon (TOC) (A), labile carbon (C_L) oxidizable with $KMnO_4$ (B), cold water extractable organic carbon (CWEOC) (C), hot water extractable organic carbon (HWEOC) (D), with dependence on a soil depth in the agroecosystem with a conventional tillage
$P < 0.05$

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to do in a given tillage system to a depth of the soil processing only in a short time period.

Even, if another tillage system is currently used on the given field, due to the stability of organic substances in the soil, it is also necessary to know the previous interventions into the soil. In the past, the conventional tillage system was used such a standard and its effect on the soil continues until today.

In the conventional tillage system, the differences between the depths are not so marked (Figure 7), which points to an equal distribution of the carbon in a given depth. Similarly, as in the reduced tillage system, the differences in C_L are also slightly larger than those of CWEOC and HWEOC. The mentioned Figures 6 and 7 prove that more marked differences in the contents of TOC and its labile fractions in relation to the tillage system were not observed. Therefore, it is rather a various distribution of relatively equal carbon contents in different depths of the soil profile. After the ploughing, the content of organic carbon at a depth of 0.30–0.45 m, mainly of its labile fractions, increases (Koch & Stockfisch 2006), and the reduced tillage does not contribute to an overall increase of organic carbon in the soil, but only to its different redistribution in the soil profile (Baker et al. 2007; Jantalia et al. 2007).

The above confirms an initial assumption and a correct decision for the selection of the indicators for a determination of the stability of organic substances from the labile parameters since the stabile components do not respond to changes in a short time period and they are rather typical characteristics of the given soil type.

CONCLUSION

The choice of a suitable indicator of the stability of organic carbon in the soil is influenced not only by a land use or soil depth, but it dependents also on the stabilization mechanisms itself. Indicators can be determined as general or specific for concrete use or conditions, which means less or more accurate. The HWEOC can be considered a general indicator of the stabilization of organic carbon for all studied land uses, but it can also be a specific indicator for some of them. It appears to be the most suitable for the forest ecosystem and, in the case of agroecosystem, for the reduced tillage at a depth of 0.0–0.1 m and for the conventional tillage at a depth of 0.0–0.3 m. In terms of the time and financial demands of its determination, the primary parameter (HWEOC) is preferred to secondary ones (CPI, LI_C). However, it is clear that each generalization means a decrease in the sensitivity of each indicator. It is appropriate to take into account the stabilization mechanism itself since its dominance is significantly influenced by the soil type. HWEOC was a specific indicator dominantly for chemical stabilization and N_L for physical one; however, both dominantly for a depth of 0.0–0.1 m, but the C_L is the most suitable for a depth of 0.0–0.3 m. Therefore, it is more appropriate to prefer the indicators that are recommended for a specific land use, soil management system or depth.

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