How to measure soil quality? A case study conducted on cropland in the Czech Republic

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Abstract: This work presents the advantages and risks of selected soil quality criteria using data from the monitoring of agricultural soils in the Czech Republic. Soil samples were taken from 71 sites covering various soil types. Basic soil parameters and mid-infrared spectra were measured. Indicators describing the quality of soil organic matter (SOM), and soil were calculated. The results show that soil types differ significantly in the qualitative indicators of soil organic matter. More acidic soils with lower clay content contain lower proportions of aromatic and higher proportions of aliphatic organic compounds than neutral soils with higher clay particles content. These soils differ little in total carbon content and C/N ratio but considerably in C/clay ratio. Cambisols are the least degraded soils in the Czech Republic in terms of C/clay ratio, which is controversial in many respects. The results indicate that more aliphatic organic matter is important for the SOM content in the upper part of the agricultural soil, and more aromatic organic matter is mainly bound to the clay fraction. The results raise questions about the suitability of uniform C/clay target values proposed in European legislation as a criterion for assessing soil degradation due to carbon loss.

Keywords: agricultural soils; infrared spectroscopy; soil organic carbon; soil texture

In recent years, more and more attention has been focused on soils, their sustainable use, quality and fulfilment of various functions, their ability to sequester carbon and generally on their health. Soil health was defined as the capacity of a living soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health (Doran et al. 1996).

As is clear from the definition, soil health cannot be measured directly but through indicators that are measurable properties of soil or plants providing clues about how well the soil can function. One group of indicators is evaluating soil quality with focusing on soil productivity. The second group contains indicators evaluating the soil ecosystem services including chemical, physical and biological soil properties, erosion processes, water purifica-

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tion ability, toxicity, and the effect on human health (Doran 2002). The selection of parameters that can adequately describe soil quality and at the same time are available, easily measurable and comparable across European countries or throughout the world is related to this effort. As part of the EJP SOIL project, a survey was conducted (Pavlů et al. 2021b; Cornu et al. 2023), within which it was found that the most commonly measured and available soil parameters across European countries include carbon concentration, pH in aqueous extract, and soil texture. But it also turned out that a number of very useful parameters are monitored significantly less. They are, for example, soil structure/aggregate stability related to porosity, water retention and resistance to erosion or bulk density and related information on carbon stocks in the soil, important for calculations of carbon cycle fluxes and balances at different spatial scales. Another example is soil organic matter quality as important characteristic of the soil both in relation to vegetation and the stabilization of carbon in the soil.

Studying of the quality of organic matter in the soil has undergone considerable development in recent decades. In the majority of current research, the assessment of the quality of organic matter based on its fractionation into humic acids and fulvic acids, as described for example by Stevenson (1994) or variously by Piccolo (2002), is rejected (e.g. Lehmann & Kleber 2015). But there are still a number of current works in which this fractionation is used or further developed (e.g. Machado et al. 2020; Jamroz & Jerzykiewicz 2022; Wang et al. 2023). More commonly, however, soil organic matter (SOM) is divided and evaluated based on the representation of particulate organic matter (POM) and mineral-associated organic matter (MAOM), which differ from each other in terms of origin, stability, and content of some elements and compounds (e.g. Zimmermann et al. 2007; Lavallee et al. 2020; Angst et al. 2021). POMs are small fragments of decomposing, mainly carbon-rich plant residues with a lower N content and a relatively short life (several years to decades). MAOM is organic matter transformed by micro-organisms with a higher N content than POM, which is protected from mineralisation by association with mineral particles and therefore persists in the soil for tens to thousands of years. Commonly used indicator of SOM quality - ratio of organic carbon and nitrogen concentration (C/N) - can in a way also describe/ distinguish nitrogen-rich organic matter and SOM for example of plant origin, and poorer-in-nitrogen substances in mineral soils (Amorim et al. 2023).

Indirect methods are gaining ground in soil science. The advantage of infrared spectroscopy is the speed of analysis/retrieval of the spectrum, from which several soil parameters can be derived simultaneously. In the case of SOM, the near-infrared region is often used for calculating the SOM content (McCarty et al. 2002; Viscarra Rossel et al. 2006; Gholizadeh et al. 2018, 2021), and the middle infrared (MIR) region for SOM quality (particularly Fourier transformed infrared spectroscopy - FTIR; Haberhauer et al. 1998; Artz et al. 2006; Leue et al. 2010; Pavlů et al. 2023b). MIR spectra can provide information about the presence of various organic components in soil (aromatic or aliphatic components, polysaccharides, lignin components), about their proportion in organic matter, and about their effect on SOM affinity to water. In addition, these spectra also provide information about the mineral composition of the soil, for example about the presence of certain minerals as carbonates, secondary silicates or quartz (Madejová 2003; Pavlů et al. 2023a). The disadvantage of this method is the frequent overlapping of spectral bands in a complex mixture of materials such as soil. MIR spectra are not quantitative and therefore various conversions must be used to obtain quantitative data, which can lead to poorer interpretability of the results.

Bispo et al. (2017) state that the wide range of methods and ways to assess the quality of SOM, as well as the lack of standardised methods, could be a barrier to its systematic inclusion in soil monitoring and databases. This is not the only reason why soil assessment is trying to move in a different direction. One way of assessing soil quality, rather than SOM quality, using available and extended data is the ratio of soil organic carbon to clay-sized particles (C/clay). It has been selected as an indicator of organic carbon status in managed mineral soils at the European Union level, to be used within the framework of the European Soil Monitoring Law proposal (Rabot et al. 2024). A ratio of 1/13 was proposed as a determining value for dividing soils into healthy and degraded categories. Although the Soil Monitoring Law refers to it as a "loss of soil organic carbon" indicator, the C/clay ratio was originally developed as an indicator of soil structural quality, because the fine fraction (clay or silt + clay) was observed to contribute to soil organic carbon protection either directly via organomineral interactions, or indirectly via the formation of aggregates (von Lützow et al. 2006).

However, the aggregation and binding of SOM are not only influenced by the presence of alumosilicates and oxides in the clay fraction of the soil but also by the properties of the organic matter itself, its hydrophobicity or hydrophilicity given by the ratio of aliphatic and aromatic components. A higher proportion of hydrophobic SOM leads to greater stability of soil aggregates in sandy loam soils (Thai et al. 2022), thus protecting the soil SOM they contain. Enclosed and physically protected soil carbon within soil aggregates contributes to its long-term fixation in the soil (Schmidt et al. 2011; Vancampenhout et al. 2012).

The question of which of the indicators is suitable for describing soil quality remains. We are aware that this study evaluates only selected indicators of soil quality and neglects other equally important ones (production capacity, nutrient supply, mineralogical composition of the soil, erosion, contamination, biodiversity, etc.). We are also aware of the considerable influence of management measures on the qualitative parameters of soil in the broadest sense of the word (e.g. Bai et al. 2018; Prudil et al. 2023; Nazaries et al. 2021). We focus mainly on parameters related to SOM as a number of supported measures on agricultural land are linked to it. So is the quantity of SOM more important, or the quality/stability of SOM in the soil environment, or its effect on soil structure, and how are all these indicators related? The aim of this study is to clarify some relationships between the quality of SOM and the content of clay particles using classical analytical methods and FTIR spectroscopy. This should not only contribute to the discussion of the use of the C/clay ratio as an indicator of soil health and its limitations, but also demonstrate the potential of non-destructive spectroscopic methods in assessing soil properties.

MATERIAL AND METHODS

For the purposes of this research, 71 selected monitoring sites (Figure 1) of the Central Institute for Supervising and Testing in Agriculture were used. The entire set of monitored sites for the Basal Monitoring of Agricultural Soils of the Czech Republic was created in 1992 and consists of a network of 190 sites maintaining the proportion of various soil types corresponding to the occurrence of these soil types in the Czech Republic. Sites are defined as rectangles with side lengths of 25×40 m and with a total area of 1 000 m². One-time samples were taken during the excavation of the soil probe for soil description and classification (according to the World Reference Base for Soil Resources; IUSS Working Group WRB 2022) and are used to determine selected physical and chemical properties of the soils. Monitoring of the content of available nutrients and soil reaction takes place in six-year intervals. Selected 71 plots are situated on land conventionally farmed by local farmers. The majority of crops in crop rotation were cereals (45.5% - mainly winter wheat, winter barley,

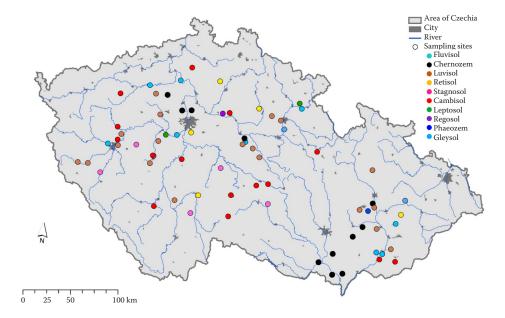


Figure 1. The map of sampling sites distribution in the Czech Republic (Czechia) with colour differentiation of Reference Soil Groups (IUSS Working Group WRB 2022)

and spring barley). Winter canola constituted 15.9% of the crop rotation, with other crops such as alfalfa, clover, potatoes, beet, silage maize, and grain maize making up a smaller proportion. This crop distribution is consistent with conventional farming practices in the Czech Republic.

At each of the selected sites, three soil samples were collected from a depth of 30 cm in 2023. Three points (distance 20 m from each other) were picked. Each point was sampled seven times and composite samples were prepared (3 composite samples from each of 71 sites make 213 samples in total). Samples were air-dried in a forced-air oven at 40 °C, and then the samples were ground and sieved for particles < 2 mm. Soil texture was determined using the pipette method (ISO 11277 2020). Soil pH in water and 0.01 M CaCl₂ extract (pH_{H₂O}, pH_{CaCl2}) were determined potentiometrically using an ion-selective electrode. For organic carbon (C_{ORG}) and total nitrogen (N_{TOT}) contents determination, the samples were further sieved for < 0.4 mm particles. C_{ORG} and N_{TOT} contents in soils were subsequently determined using the CNS analyser Elementar Vario Macro (Elementar Analysensysteme, Germany) after carbonate digestion with HCl. The ratios of CORG to N_{TOT} and C_{ORG} to clay content were calculated and hereafter referred to as C/N and C/clay, respectively.

Part of the sieved samples (< 2 mm) was ground to analytical fineness to measure the infrared spectra using the diffuse reflection technique and instrument Nicolet iS10 (Thermo Fisher Scientific Inc., USA). The spectral range was 4 000–400 cm⁻¹. Before the measurement, the samples were not diluted with KBr or otherwise modified. The gold mirror was used as a background reference. 64 scans with a resolution of 4 cm⁻¹ and spectra conversion to Kubelka–Munk units were applied. OMNIC 9.2.41 software (Thermo Fisher Scientific Inc., USA) was used for spectra analysis. Well-defined bands of soil component functional groups were identified in the spectra, and their reflectance was used for subsequent calculations.

To quantify selected spectral bands, their reflectance was expressed as a percentage of the summed reflectance. The aromaticity index (iAR) was calculated according to the reflectance of aliphatic C-H bands in the range 2 950–2 820 cm⁻¹ (AL) and aromatic band at 1 520 cm⁻¹ (AR) (iAR = AL/(AL + AR); Cunha et al. 2009). A higher index value indicated a lower aromaticity of the soil organic matter. The use of this index is limited in soils with a higher carbonate content, where the peak around 1 520 cm⁻¹ can be covered by a broad and intensive

carbonate band. However, only soils from 5 sampling sites contained enough carbonates to limit the use of this index. These samples were excluded from relevant calculations and statistical analyses.

Potential wettability index (PWI) connecting information about organic matter quality and its effect on soil structure was evaluated according to the adsorption band of the alkyl C-H groups – A (in range 2 950–2 820 cm $^{-1}$), indicating the relative hydrophobicity, and adsorption band of the C=O groups – B (in the ranges 1 740–1 698 and 1 640–1 600 cm $^{-1}$, respectively), which is ascribed to hydrophilicity. PWI was calculated as a ratio by summing up the intensity of the C-H and C=O groups (PWI = A/B) (Ellerbrock et al. 2005). A higher index value indicated a lower wettability of the soil.

Ratio of band in range 1 640–1 600 cm⁻¹ to band around 1 520 cm⁻¹ was used as an indicator of organic matter transformation/decomposition (decomposition index – DI; Haberhauer et al. 1998). A higher index value indicated a higher proportion of oxygen containing functional groups what documents a higher level of transformation of aromatic substances based on lignin. For the same reason as iAR, also this index is inappropriate for strongly carbonatic soils.

The Statistica software (Ver. 13.5.0.17) (TIBCO Software Inc., USA) was used to perform statistical analyses. First, the normality of all data sets was tested (Kolmogorov-Smirnov test). Outliers (clay content > 50%) were excluded from the dataset. The basic statistical parameters, such as the mean and coefficient of variation (the ratio of the standard deviation to the mean; the statistical measure of the dispersion of data points in a data series around the mean), were computed. A one-way analysis of variance (ANOVA) was used to analyses the differences between soil types. A Fisher LSD test was computed for the categorical variables (95.0% LSD (least significant difference)). Basic relationships between the soil properties were assessed by correlation analysis. The correlation coefficient matrix was expanded with information about significance at different probability levels. Based on the correlation analysis results, which showed relationships between the studied variables, values of the basic soil characteristics and spectral parameters were treated with a factor analysis.

RESULTS AND DISCUSSIONS

Basic soil characteristics. Soils of studied sampling sites were sorted based on Reference Soil Groups

(RSG; IUSS Working Group WRB 2022). Figure 2A shows the distribution of RSG in the sampling sites set. The most represented RSG are Luvisols (19 sites) and Cambisols (15 sites), and the least represented RSG are Leptosols and Gleysols, both in 2 sites, and Regosols and Phaeozems each with 1 site only. Because of limited data, the four last mentioned RSG were excluded from analyses of variance identifying differences in soil conditions between RSG. Figure 2B presents the distribution of soil texture classes in the dataset. The texture of most the soils appertains to variants of loam. Clay soils are on three sites where clay content exceeds 50%, while in all other cases, it ranges from 6% to 39%. Significantly

highest clay content among more represented RSG was found in Fluvisols (mean \pm standard deviation (SD): $25.3\pm6.4\%$) and the lowest in Cambisols (14.7 \pm 7.8%). However, it is important that the variability of the values is high as it is visible in a high value of SD and a high coefficient of variation – 53.1% (Table 1) in the Cambisols dataset (even after discarding the site with the highest clay content (50.6%) due to compliance with the condition of normality of the data for the analysis of variance). Values of pH range from 5.4 to 7.9 in the case of pH_{H2O} and from 4.9 to 7.4 in the case of pH_{CaCl2} in the whole dataset. The lowest pH (pH_{H2O}; pH_{CaCl2}) values were found in Stagnosols (6.4 \pm 0.37; 5.8 \pm 0.32) and Cambisols

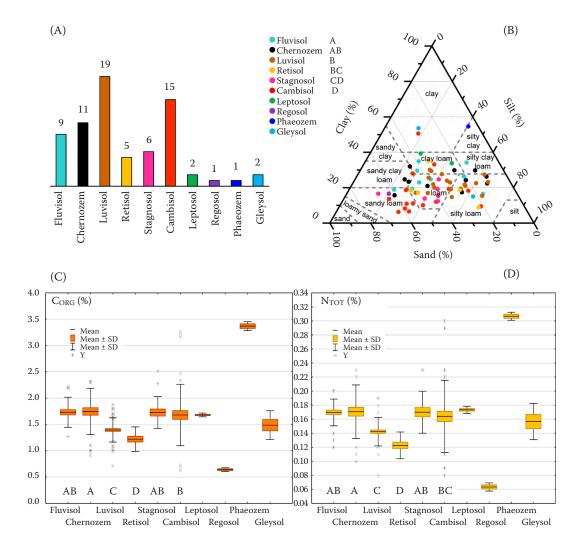


Figure 2. The distribution of Reference Soil Groups (RSG) in sampling sites set (A) and basic soil characteristics distribution in dataset with displaying of significant differences between more represented RSG tested by ANOVA (95% LSD); different letters represent significantly different contents of clay (B) (also shown soil texture classes according to the position of individual sampling sites in texture triangle), of organic carbon (C_{ORG}) (C), and of total nitrogen (N_{TOT})(D) SD – standard deviation; SE – standard error (SE); Y – values outside the \pm SD range

Table 1. Breakdown table of descriptive statistics (sorted by soil type)

		Clay co	Clay content (%)			Cog	Corg (%)			$N_{ m T}$	N _{TOT} (%)			C/ci	C/clay (-)	
KSG	mean	и	SD	CV	mean	и	SD	CV	mean	и	SD	CV	mean	и	SD	CV
Fluvisol	25.27	27	6.368	25.20	1.732	27	0.2865	16.54	0.170	27	0.0191	11.27	0.0736	27	0.02547	34.60
Chernozem	24.24	33	4.947	20.40	1.747	33	0.4410	25.24	0.171	33	0.0383	22.46	0.0740	33	0.02099	28.35
Luvisol	22.11	22	4.425	20.01	1.395	22	0.2305	16.52	0.142	22	0.0204	14.31	0.0664	22	0.01938	29.21
Retisol	19.63	15	8.844	45.05	1.220	15	0.2339	19.18	0.123	15	0.0191	15.55	0.0714	15	0.02447	34.27
Stagnosol	17.01	18	4.167	24.50	1.726	18	0.3041	17.61	0.170	18	0.0299	17.59	0.1061	18	0.02724	25.67
Cambisol	14.70	42	7.801	53.08	1.680	45	0.5841	34.78	0.164	45	0.0513	31.29	0.1320	45	0.07477	56.63
Leptosol	34.80	9	4.930	14.17	1.680	9	0.0358	2.13	0.173	9	0.0052	2.98	0.0491	9	0.00670	13.65
Regosol	16.60	3			0.640	3	0.0361	5.63	0.063	33	0.0058	9.12	0.0386	3	0.00217	5.63
Phaeozem		0			3.367	3	0.0850	2.53	0.307	3	0.0058	1.88	0.0617	3	0.00156	2.53
Gleysol	15.00	3			1.487	9	0.2741	18.43	0.157	9	0.0258	16.48	0.0582	9	0.02909	49.96
All groups	20.90	204	7.408	35.44	1.596	213	0.4740	29.70	0.158	213	0.0412	26.05	0.0849	213	0.04764	56.12
		iA	iAR (–)			ΡW	PWI (-)			D	DI (-)			C/I	C/N (-)	
	mean	и	SD	CV	mean	И	SD	CV	mean	и	SD	CV	mean	и	SD	CV
Fluvisol	0.0140	26	0.00268	19.11	0.0208	27	0.0044	20.99	1.551	23	0.1157	7.46	10.39	27	1.149	11.06
Chernozem	0.0123	25	0.00223	18.08	0.0184	33	0.0046	25.12	1.561	24	0.0984	6.30	10.30	33	0.863	8.38
Luvisol	0.0126	54	0.00272	21.51	0.0185	99	0.0041	21.96	1.467	54	0.0914	6.23	6.87	22	0.752	7.62
Retisol	0.0136	15	0.00258	18.98	0.0203	15	0.0037	18.15	1.407	15	0.0927	6.59	10.05	15	0.837	8.34
Stagnosol	0.0182	18	0.00643	35.28	0.0271	18	9600.0	35.57	1.397	18	0.0678	4.86	10.46	18	0.951	60.6
Cambisol	0.0185	45	0.00552	29.90	0.0272	45	0.0083	30.35	1.474	45	0.1895	12.85	10.33	45	0.915	8.85
Leptosol	0.0105	3	0.00056	5.30	0.0203	9	0.0057	28.02	1.536	3	0.0191	1.25	9.78	9	0.079	0.80
Regosol	0.0086	3	0.00057	6.59	0.0132	3	0.0000	6.46	1.346	3	0.0092	89.0	10.31	3	0.427	4.14
Phaeozem	0.0186	3	0.00026	1.42	0.0253	3	0.0002	09.0	1.915	3	0.0107	0.56	11.03	3	0.105	0.95
Gleysol	0.0158	9	0.00368	23.32	0.0230	9	0.0048	20.91	1.524	9	0.1010	6.62	9.65	9	0.351	3.64
All groups	0.0148	198	0.00472	31.92	0.0217	212	0.0070	32.25	1.487	194	0.1423	9.57	10.18	213	0.889	8.73

RSG – reference soil groups; n – number of samples (usually 3 composite samples per location); SD – standard deviation; CV – coefficient of variation; iAR – aromaticity index; $\ensuremath{\mathrm{PWI}} - \ensuremath{\mathrm{potential}}$ wettability index; $\ensuremath{\mathrm{DI}} - \ensuremath{\mathrm{decomposition}}$ index

 $(6.5 \pm 0.85; 5.8 \pm 0.81)$. In general, they can be classified as weakly acidic, although some individual values fall into the acidic or neutral categories. The highest pH values were found in Chernozems $(7.3 \pm 0.53; 6.8 \pm 0.54)$ and Fluvisols $(7.5 \pm 0.72; 6.9 \pm 0.81)$, and it is possible to classify them as weakly alkaline (although individual values reach the weakly acid category).

Box plots of Figure 2C and 2D display the distributions of C_{ORG} and N_{TOT} contents in studied soils. Generally, the distributions of both element contents are almost identical which is understandably related to their identical origin in SOM. The lowest C_{ORG} and N_{TOT} contents in the whole dataset are in Regosol and the highest in Phaeozem, but similar individual values were found also in Cambisols dataset. Most values are within the range 1–2% of C_{ORG} and 0.10–0.22% of N_{TOT} . A significant difference was found between more represented RSG. The highest contents of C_{ORG} and N_{TOT} were found in Chernozems (1.75 ± 0.44 % and 0.17 ± 0.04 %), and the lowest was in Luvisols (1.40 ± 0.23 % and 0.14 ± 0.02 %) and Retisols (1.22 ± 0.23 % and 0.12 ± 0.02 %).

Soil organic matter quality and soil health indicators. Measured diffuse reflectance infrared (DRIFT) spectra of soils are shown in Figure 3, and important spectral bands are highlighted. As was mentioned in the introduction, DRIFT spectra of soil bring information about mineral and organic components of soil. Even from a simple percentage expression of the heights of the individual bands, it is possible to infer certain properties of the soils. For example, a band around 810 cm⁻¹ belonging to quartz is relatively

most represented in Regosol at the expense of bands of clays and oxides in the range 3 600–3 700 cm⁻¹. It corresponds to the sandy texture of this RSG with a low proportion of clay fraction. The exact opposite is the case of Phaeozem. The sum of aliphatic bands percent approximately corresponds to organic matter content in soils. The lowest values are in Regosol and the highest in Phaeozem.

Differences between RSG in the representation of aliphatic and aromatic components of SOM can also be detected in this way, but the calculation of various indexes is a more accurate way how to describe the qualitative parameters of SOM. The aromaticity index describes the mutual proportion of spectral bands of aliphatic and aromatic SOM components. Identical aliphatic bands are used in the calculation of the potential wettability index. In this case, aliphatic bands are related to aromatic and oxygen-containing (carboxyl, ketone) bands (around 1 720 cm⁻¹ and in the range 1 640–1 600 cm⁻¹). The vibration of aromatic rings is generally considered to indicate hydrophobic groups as well as aliphatic groups, but aromatics can show hydrophilic properties when conjugated with C=O groups (Ellerbrock et al. 2005; Leue et al. 2010). The values of both indexes are similarly distributed between RSG (Figure 4A, C). Significantly, the highest values are in Stagnosols and Cambisols, which document the highest proportion of aliphatic SOM components there and the lower wettability of their SOM.

The ratio of the band in the range 1 640–1 600 cm⁻¹ to band around 1 520 cm⁻¹ was used by Haberhau-

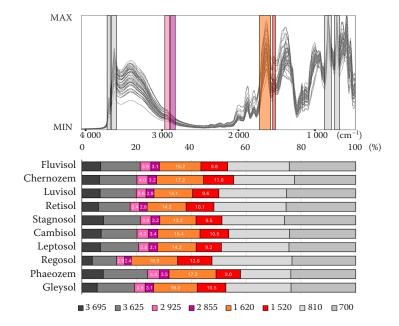


Figure 3. DRIFT spectra of soils with highlighting of spectral bands of mineral soil components (grey) and of organic soil components (coloured) (top); percentage expression of the average representation of selected bands in the spectra of Reference Soil Groups (bottom)

Wavenumbers in cm⁻¹ of the approximate centres of the spectral bands are used in the legend; white numbers show average representation of organic bands in %

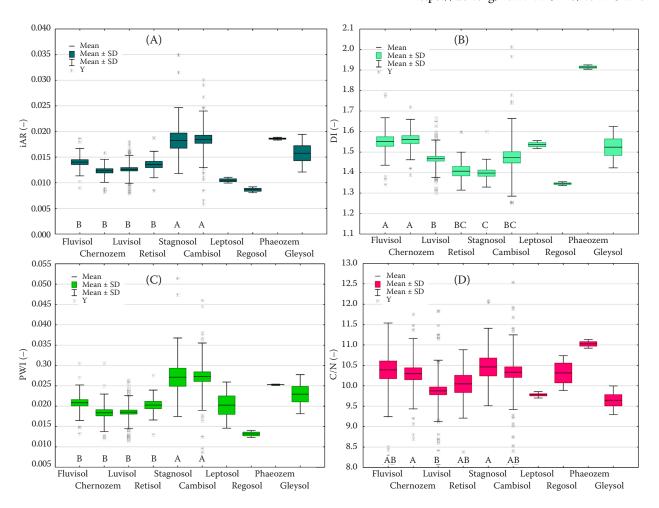


Figure 4. The distribution of soil organic matter quality indicators in the sampling sites set with displaying of significant differences between more represented RSG tested by ANOVA (95% LSD): different letters represent significantly different values of aromaticity index – iAR (A), decomposition index – DI (B), potential wettability index – PWI (C), and organic carbon to total nitrogen ratio – C/N (D)

SD – standard deviation; SE – standard error (SE); Y – values outside the ±SD range

er et al. (1998) as an indicator of organic matter decomposition in litter layers of forest soils. The band of amides overlaps the aromatics band around 1 520 cm⁻¹. These bands are sometimes separated into two individual peaks around 1 540 cm⁻¹ for amides and 1 512 cm⁻¹ for aromatics (Pavlů & Mühlhanselová 2018), but in the spectra of mineral soil mostly merge into one peak with a prevailing band of aromatics (discussed later). Therefore, this index describes the ratio of two spectral bands both including vibration of aromatic SOM components, but the one around 1 620 cm⁻¹ also captures oxygen substitution groups. In addition, it is known that organic matter of plant origin contains lignin as one of the dominant aromatic components (Xu et al. 2024). The spectrum of lignin has a dominant aromatic band at 1 512 cm⁻¹, while the "breathing" vibration of the benzene ring (1 600 cm⁻¹) is less intense (Agnelli et al. 2000). Lignin polymer has undergone microbial degradation from plant residues to small molecules and has most likely been oxidized, as it needs to undergo depolymerization before reacting with mineral phases (Thevenot et al. 2010). Lower DI (thus a higher relative proportion of 1 520 cm⁻¹ band) can indicate a higher presence of less transformed plant-derived components of SOM. Index DI differs from previously mentioned indexes in the distribution between RSG. While in the previous indexes, there was no significant difference between Fluvisols, Chernozems, Luvisols and Retisols, in this case, Fluvisols and Chernozems achieve significantly higher DI values than Luvisols and Retisols and also

than Stagnosols and Cambisols. It can, therefore, be concluded that Fluvisols and Chernozems have a lower proportion of slightly transformed plant-derived components than other RSG.

For comparison, Figure 4D shows non-spectral parameter used in SOM quality evaluation. The C/N ratio differentiates SOM on the base of N-rich components content which are understood as relatively fresh organic residues attractive for decomposition or microbially transformed organic matter. C/N values < 10 are associated with more biologically active and humus soils. The higher the C/N ratio, the higher the proportion of plant-derived SOM poor in nitrogen (Amorim et al. 2023). C/N ratio varies in a relatively narrow range from 8.3 to 12.5 (mean – 10.2; coefficient of variation -8.7%) in studied dataset. A significant difference was found between Luvisols with lower C/N (9.9 \pm 0.75) and a group of Chernozems and Stagnosols with higher C/N (10.3 \pm 0.86; 10.5 ± 0.95). These values correspond to the average for cropland evaluated within the framework of Agrochemical Testing of Agricultural Soils of the Czech Republic and belong to the medium category (C/N in range 8-11) according to Sotáková (1982). The values for topsoil horizons in the Czech Republic presented in the publication (Matschullat et al. 2018) are also in a similar range. Greater variability of the data was found within the whole of Europe, where the parameters of the geological bedrock (pH, carbonates) in combination with climatic conditions are more pronounced in the distribution.

The last calculated soil quality indicator is a ratio of C_{ORG} to clay contents. The theory of Dexter et al.

(2008) assumes that 10 g of clay enables the complexation of 1 g of organic carbon. Johannes et al. (2017) concluded that C/clay ratios of 1/8, 1/10 and 1/13 were appropriate thresholds to distinguish very good, good, moderate and degraded soil structures. Figure 5 shows, in addition to the distribution of ratios between RSGs, these recommended thresholds for soil health. Similarly, as in the case of C_{ORG} and clay contents separately, also these ratio values are the most variable in Cambisols. The coefficient of variation reaches almost 57% (Table 1). Nevertheless, the values of this ratio/indicator for Cambisols are significantly the highest of all the RSGs, followed by Stagnosols and then all the other RSG, which are no longer different from each other. It can also be stated that only Cambisols exceed the value indicating that the soil is considered healthy (has very good soil structure/ it is not degraded by carbon loss). Stagnosols reach values referred to as good soil structure, and other RSG fall into the category of degraded.

It is clear from the nature of the data ranges used to calculate the C/clay ratio that the clay content is more strongly reflected in the resulting value. Clay content ranges from units to tens of percent while the carbon content fluctuates only between 1–2%. This criterion was proposed for evaluating changes over time and between various land uses when the clay content is practically constant and the carbon content changes (Prout et al. 2022). This is consistent with the requirement to capture "soil organic carbon loss" with this parameter. Prout et al. (2022) show that normalising clay concentration provides a more meaningful separation between land uses

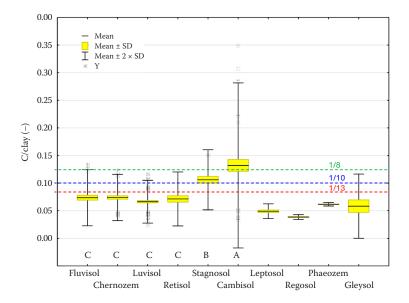


Figure 5. The distribution of organic carbon and clay contents ratio (C/clay) in the sampling sites set with displaying of significant difference between more represented reference soil groups tested by ANOVA (95% LSD) The colour dashed line indicate the thresholds reported to distinguish very good (> 1/8), good (> 1/10), moderate (> 1/13), and degraded (< 1/13) soil structures; different letters represent significantly different values of ratio; SD – standard deviation; SE – standard error (SE); Y – values outside the ±SD range

than changes in organic carbon content alone. The authors state that given the wide range of soils and land uses across England and Wales in the datasets used to test these targets, they should apply across similar temperate regions globally.

On the contrary, the results of Rabot et al. (2024) from France show that C/clay and C/(silt < 20 µm + clay) were poor indicators of the soil bulk density and aggregate stability. The organic carbon content was a much better indicator of soil structure. A number of works confirm the effect of carbon content on the stability of the soil structure (e.g. Kodešová et al. 2009; Zádorová et al. 2011; Jakšík et al. 2015; Pavlů et al. 2021a). Rabot et al. (2024) also state that by normalizing the C_{ORG} content by the fine fraction content, these indicators provide an oversimplified view of the links between CORG content and soil structure. In particular, C/clay was found to be strongly affected by soil pH, with acidic soils consistently being classified as healthy according to the threshold of 1/13, and alkaline soils often being classified as unhealthy. This is in accordance with our findings, as Cambisols and Stagnosols belong to the most acidic soils from the entire data set and are the only ones to reach "favourable" values of the C/clay ratio.

Theoretically, for soil with a clay content e.g. 25% (near to real clay content average values of Chernozems in our dataset) to be considered "very good", it would have to contain more than 3.125% of carbon, and to be considered degraded by carbon loss, the carbon content can be up to 1.9%. Brady and Weil (2008) state that the carbon content in Chernozems varies between 1-4% (in virgin prairie Chernozems, it was found to be 5.6 %) with typical values around 2.4%. In the Czech Republic, carbon content lower than 2% was documented in buried Chernozems with an estimated age covering colluvial layer of 4-7 thousand years (Zádorová et al. 2023). It is clear from this list that Chernozems, with a clay content of 25% given as an example and known as soils rich in organic matter, can hardly reach the target value of C/clay 1/13. It is even more obvious with Luvisols or Retisols with similar clay content as Chernozems but significantly lower natural content of organic carbon.

Interrelationships. Correlation analysis was used for interrelationships search between basic soil characteristics, conventionally measured indicators of soil or SOM quality, and spectral parameters of SOM (Table 2). The strongest correlation was found between PWI and iAR indexes. Both these indexes are

based on the relation of selected spectral bands where one group of bands belongs to aliphatic components (C-H bonds) of SOM, and the other includes spectral bands of aromatics. PWI index is calculated from bands around 1 620 cm⁻¹ which includes vibrations of C=O bond in various substitution groups on aliphatic chains as well as on aromatics and of C=C bond in benzene ring. The iAR index calculates with a band of aromatics around 1 520 cm⁻¹. This band includes (except the band of C=C bond in the benzene ring) bands of amides. The high correlation of these indexes and also a correlation of the 1 520 and 1 620 bands percentages in spectra indicate the dominant representation of C=C bands of aromatics in these peaks.

The second strongest correlation was found between C_{ORG} and N_{TOT} , which is explicable by the same source of these elements and similar proportions in SOM (low variability of C/N) of this dataset mentioned above.

Significant dependences between individual variables were found also in a number of other cases. Spectral parameters correlate with basic soil properties and with indicators of soil and SOM quality. Interrelationships can be assessed more clearly using multivariate statistical analyses. Factor analysis was used in this work for this purpose. Variables PWI, N_{TOT} and (%)1520 were excluded from the analysis due to data collinearity (correlation with variables iAR, C_{ORG} and (%)1620, mentioned above) causing instability of the model. Soil quality or, more precisely, "loss of soil organic carbon" indicator - C/clay was excluded from factor analysis as we can answer the question of what affects the resulting value of this parameter. Two factors (together explaining 63.2% of the data variability after varimax rotation) were extracted (Figure 6A). The first factor (F1) is represented by high loadings of CORG content, C/N ratio, iAR, and percentage of band 2 925 cm⁻¹. The second factor (F2) includes loadings of clay content, DI, and percentage of band 1 620 cm⁻¹. The first factor could be explained as an effect of plant-derived organic material with the lower portion of nitrogen and a higher portion of aliphatic components of SOM (lower aromaticity) on the content of C_{ORG} in soil. The second factor indicates a more stable component of organic matter bound to the clay fraction of the soil. Figure 6B shows distribution of factor scores and differentiates soil samples according to C/clay ratio and soil categories coming from this ratio. It can be seen that the best/least degraded sites

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Fable 2. Correlation matrix

C/clay 0.301 -0.733** 0.358*** 0.012 0.347** -0.1350.492** 0.619*** 0.666** .429** 0.052 0.365** 1.000 204 204 185 185 185 185 203 189 204 204 204 0.197*** -0.045).352*** 399 0.365*** -0.0470.172** -0.234* 1.000 C/N0.020 0.054 0.450213 0.001 213 213 204 194 194 198 194 194 .0.371** 0.416***).561*** -0.099-0.1360.438*** -0.0400.666*** 0.995 -0.436** 0.501*** 1.000 0.399198 189 194 189 198 198 198 194 194 198 198 194 198 -0.069-0.160* 0.619*** 0.433*** -0.1170.396 0.430** 1.000 0.352** -0.402* PWI 198 212 203 212 194 194 194 194 -0.301*** 0.364*** 0.344** 0.357*** -0.067-0.117-0.0450.494*** 0.289 -0.0403.451** 000.1 194 194 194 194 194 194 185 185 194 194 194 194 194 DI -0.426*** (%)29250.266*** 0.492*** 0.338 0.289 0.553** 0.130 1.000 0.396** 0.438**0.172* 185 194 194 194 194 194 185 194 194 194 194 194 194 (%)16200.751*** -0.160* -0.135-0.0471.000 0.338** 0.079 0.083 185 194 185 194 194 194 194 194 194 194 194 194 (%)15200.751*** 0.344** -0.258** -0.226* 1.000 -0.0990.221*0.130 0.052 194 0.001 194 194 194 185 185 194 194 194 194 194 194 -0.258** 0.347*** 0.494*** 0.430 0.501*** 3.959 1.000 0.083 0.553** 0.197* 213 213 204 194 194 194 198 194 0.429*** 0.451*** 0.561 *** 0.959 0.450**1.000 -0.226* 0.079 0.557*204 194 194 194 194 0.358*** 0.364** -0.229* 0.221** -0.0260.433** -0.202* 1.000 194 0.266 194 212 0.416** 204 194 194 -0.348*** -0.371*** .0.188** -0.426** -0.052-0.0640.012 1.000 0.020 213 212 204 198 194 194 194 194 -0.733*** -0.402*** .0.436*** -0.383*** 0.515*** -0.144-0.234* 0.171* 1.000 0.079 185 185 185 203 189 204 204 185 \geq \approx R \mathcal{B} \approx \geq \approx \geq \approx \aleph \geq \aleph \aleph \geq \aleph \geq \geq (%)1620Variable (%)1520(%)2925C/clay NTOT Corg Sand PWI C/N Clay **IAR** Silt Ξ

R – correlation coefficients; N – the number of elements of the statistical data set (213 soil samples, outliers of clay content or spectral parameters affected by high carbonate content are excluded in appropriate variables combination); correlations affecting factor analysis are in bold; the designation (%)1520, (%)1620, and (%)2925 corresponds to the percentage representation of the respective bands in the spectrum); *, ***, ***significant at 0.05, 0.01, and 0.001 probability level, respectively

by soil carbon loss are those with lower clay content and higher contents of less aromatic organic matter. According to Figures 6C and 6D, this corresponds to Cambisols and Stagnosols. On the contrary, the most aromatic and clay-bound organic components are present in Chernozems and Fluvisols (Figure 6D).

Pavlů et al. (2023a, b) found similar noticeable differences in the qualitative composition of SOM between different soil types. Chernozems differ from Cambisols in the aromaticity of organic matter and DI. Aromatic compounds frequent in Chernozems are less mobile because of their hydrophobic character which leads to the formation of stable microaggregates and selective retention on mineral surfaces

(Feng et al. 2005; Bi et al. 2013) and nitrogen-rich, microbial-derived biomolecules like aminosugars/ acids are also selectively bound to mineral surfaces (e.g. Kopittke et al. 2018). Amorim et al. (2022) prove preferential accumulation of N-rich SOM in the soil clay fraction. Moreover, the same work points to the fact that the C/N ratio is related to SOM size distribution. Relatively high C/N ratios are typical in particulate SOM, which commonly comprises plant tissues only partly decomposed, and thus more labile. Conversely, clay-sized SOM almost always has lower C/N ratios but tends to be more stable than sand-sized SOM. Iron oxides play an important role in aliphatic components of SOM fixation in the case

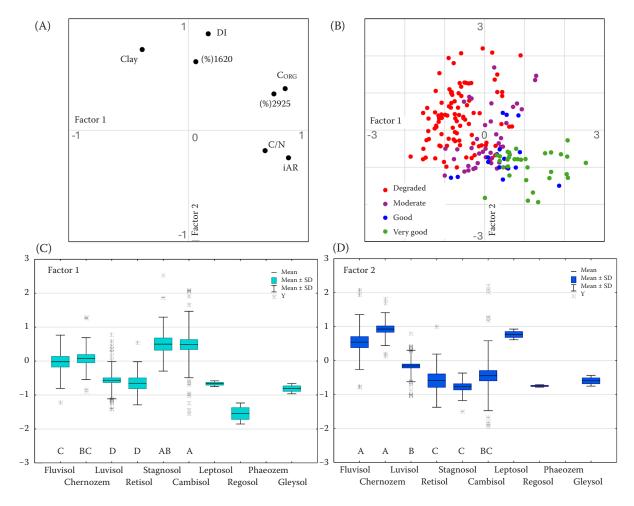


Figure 6. Results of factor analysis: factor loadings of selected soil properties after varimax rotation (A) (the designation (%)1620 and (%)2925 corresponds to the percentage representation of the respective bands in the spectrum); factor scores with the sorting of points into groups according to the value of the ratio of carbon and clay contents and the respective class of soil quality (B); the distribution of factor 1 (C) and factor 2 (D) scores in the sampling sites set with displaying of significant differences between more represented Reference Soil Groups tested by ANOVA (95% LSD)

Different letters represent significantly different values; SD – standard deviation; SE – standard error (SE); Y – values outside the ±SD range

of Cambisols. Polysaccharides and proteins are also selectively preserved in organo-metallic complexes (Nierop et al. 2005; Tonneijck et al. 2010). It was proved by Yang et al. (2024) for tropical Ferralsols that various Fe/Al phases, depending on their crystallinity, exhibit preferences for binding specific organics and preserving them in these organo-mineral associations for the long term. Short-range ordered oxide phases tend to prefer lignin-like and tannin-like compounds while crystalline phases prefer aliphatic-like compounds. This finding also indicates the potential of aliphatic C compounds in stabilizing SOM within mineral phases. Moreover, the physical way of SOM stabilization by decreasing wettability and increasing stability of soil aggregates (Thai et al. 2022) is crucial for the hydrophobic components of SOM. Xu et al. (2024) state that the hydrophobic alkyl carbon and aromatic carbon are considered as relatively stable C. From the works of Pavlů et al. (2023b) and Yang et al. (2024), it is also clear that land use or soil management can affect the total amount of carbon in the soil but will not fundamentally affect the distribution of fixed components. The distribution is controlled mainly by the mineralogy of the soil.

It is therefore obvious that it is impossible to say which part of the organic matter in the soil is more stable, but the results of the factor analysis show that more aliphatic organic matter (probably mainly of plant origin) is more important for the amount of SOM in the tested upper part of the agricultural soil, and the clay fraction binds mainly more transformed and aromatic organic matter. Even this selectivity of SOM binding to the clay fraction may disqualify the C/clay ratio as a single indicator of soil quality.

CONCLUSION

The results clearly show that the qualitative parameters of SOM, soil texture and mineralogical composition play a very important role in the fixation of carbon in the soil. All the listed properties together define individual soil types and their variability. It is, therefore, difficult to set some simple and generally valid criterion that would be able to determine whether a soil is healthy or not. Although the proposed C/clay ratio can capture soil degradation or recovery of a particular soil or of soils in particular conditions well, either from the point of view of carbon loss or from the original definition based on the stability of soil structure over time, it is not possible to link it to the same target value for all soil types. Spectral

parameters are a relatively simple way to describe the qualitative composition of SOM and could be included in routine monitoring of soil properties. Although not as accurate as direct analytical methods, they can help to assess changes in SOM over time. And even if the quality of organic matter captured appears to depend mainly on the mineralogy of the soil, it may be interesting to monitor it over longer time intervals, for example in relation to other degradation processes that also affect the mineralogy, such as acidification or salinisation of soils.

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