Spectroscopic characterization of soil organic matter quality in intensively used Chernozems

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Chernozems are considered highly productive soils, which are strongly affected by intensive management, erosion processes, and degradation. Three different soil types were classified according to Němeček et al. (2011) along the transect in the field – Calcic Chernozem (control site); Calcaric Regosol (erosion site); and Calcic Chernozem Colluvic (accumulation site). Soil organic matter quality was evaluated using infrared and UV-VIS spectroscopic methods. Humic acids (HAs) were isolated from all three soil samples according to the standard IHSS method. DRIFT spectroscopy (diffuse reflectance infrared Fourier transform spectroscopy) was applied to evaluate HAs chemical composition and hydrophobicity. Both parameters can better characterize HAs stability against microbial degradation, reactivity, and wettability. Results showed that the humification degree was the highest in Calcic Chernozem Colluvic (accumulation site). Less humified were HAs in Calcaric Regosol (erosion site). Higher content of aliphatic labile and hydrophilic groups was in HAs from Calcaric Regosol (erosion site). The content of aromatic stable and resistant components was higher in HAs from Calcic Chernozem (control site) and Calcic Chernozem Colluvic (accumulation site). The highest hydrophobicity index was in HAs from Calcic Chernozem Colluvic (accumulation site). The HAs chemical composition and hydrophobicity are crucial in soil productivity and organic matter stability in a changing environment.

Keywords: humic acids, chernozem, DRIFT and UV-VIS spectroscopy

1   Introduction

The chemical composition of soil humic acids (HAs) is given by plant- and microbial-derived components and is also influenced by climatic conditions, soil types, land uses, and fertilizing systems. As the stable fraction of SOM, HAs directly influence carbon storage, structure, aggregates stability, nutrition and water regimes, and overall agroecosystem stability. Non-destructive spectroscopic characterization of HAs arises from electron transitions within molecules. Broad absorption bands are usually observed in UV-VIS and infrared spectral ranges due to the contribution of vibrational and rotational energy levels. The principal characteristics of an absorption band are its position and intensity and both techniques are frequently used for the determination of optical curves and important indexes calculation (Chen et al., 1977; Kumada, 1987; Ellerbrock et al., 2005; Demyan et al., 2012). According to Del Vecchio and Blough (2004) and Pospíšilová et al. (2016), spectroscopic characterization in UV-VIS spectral range is related to the degree of condensation of the aromatic carbon network, carbon content, and molecular weight.

As quoted by these authors spectroscopic methods are frequently used to document the changes in soil organic matter composition after amending soil with different exogenous organic materials (e.g. digestate, compost, biochar etc.). Also, Xie et al. (2022) showed that UV-VIS and Vis-NIR spectroscopy can be very effective in the prediction of organic carbon content and other soil properties. HA, the main component of soil humic

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substances is usually studied in the mid-and near-infrared region and different techniques are applied (e.g. diffuse reflectance infrared Fourier transform spectroscopy, golden gate single reflection, diamond attenuated total reflectance, horizontal attenuated total reflection). Chen et al. (2022) showed that Fourier-transformed near-infrared (FT-NIR) spectroscopy with a combination iPCA-LR (interval search principal component analysis algorithm with logistic regression) can provide a direct and rapid quantitative determination of soil organic matter status. IR techniques are useful because allow for collecting spectra from powdered samples with minimum preparation and disturbance. Results of HAs spectroscopic research in the IR region allow us to characterize the content of aromatic, aliphatic and other groups presented in HAs molecule. The most important are aliphatic C-H units, which control the water affinity, aggregates' stability, and rate of organic matter decomposition (Capriel et al.,1997; Ellerbrock et al., 2005; Šimon, 2008; Demyan et al., 2012). In addition, C-H units are responsible for the rate of wetting, and adsorption processes, and contribute to the organic carbon cycle in the ecosystem. Identification of stretching and bending functional groups is possible due to the given amount of energy and molecular motion. The intensity of each band is related to the charge in the electrical dipole moment of the bond during the vibration (Griffiths & de Haseth, 2007). Infrared (IR) spectroscopy is very useful for agricultural purposes because of its cost- and time-effective advantages. Today, the IR library is more available and various calibration models may be developed to predict organic carbon storage and other soil's physical and chemical properties (Viscarra-Rossel et al., 2008; Viscarra-Rossel & Bouma, 2016; Biney, 2022). On the other hand, the determination and studying of spectrally active groups in HA molecules highly depend on sample preparation and yields (Tinti et al., 2015, Pospíšilová et al., 2016). Therefore, we feel a gap in knowledge about how HA chemical composition is affected by intensive farming. The presence of aliphatic and aromatic groups directly affects HA's reactivity, wettability, and soil organic matter stability. HA properties are necessary better understand because they are a key product of humification and directly influence soil quality/health.

The main hypothesis is that humic acids' chemical composition and the presence of aliphatic C-H and aromatic groups are affected by land use, management practices, and erosion processes. As a result, changes in the chemical composition and hydrophobicity affected soil organic matter stability, hydrophobicity, wettability, and degradability.

2 Material and methods

Calcic Chernozem was investigated during 2018-2022 (locality Bošovice, district Vyškov, Czech Republic). The soil type was classified according to the IUSS Working Group WRB (2015). The investigated soil in the entire location was classified as Calcic Chernozem (control site) but due to intensive cultivation and erosion gradually degraded and was reclassified to the Calcaric Regosol (erosion site) and Calcic Chernozem Colluvic (accumulation site). The soil derives from loess and contains 22.28% sand, 46.20% silt, and 31.52% clay. The average values of the active soil reaction were 7.9 and the exchangeable soil reaction was 7.5. Soil samples were collected along with the transect-control site (C), erosion site (E), and accumulation site (A). Each soil sample was a composite of 10–15 random subsamples, which were taken from each variant from a depth of 0–10 cm. Oxidizable carbon (Cox) was determined in air-dried soil samples by wet combustion according to Nelson & Sommers (1996). The short fractionation method was used for the determination of the fractional composition of humic substances (Kononova & Belchikova, 1963). The humic and fulvic acids ratio (HAs/FAs) ratio and the absorbance ratio (Q4/6) were calculated. The absorbance ratio was calculated as the ratio between absorbance at 446 nm and 665 nm (A446/A665). Spectral properties of humic substances were measured in the mixture 1 : 1, 0.1 M NaOH + 0.1M $Na_4P_2O_7$ using UV-VIS Varian Carry 50 spectrometer with optical fibre, within a range of 300–700 nm.

Humic acids were isolated according to the IHSS standard method described by Stevenson 1982 and Hayes (1985). In short: 100 g of the air-dried soil sample, was sieved at a mesh size of 1 mm, washed with 10% HCl and stirred for 1–2 hours (decalcination process). After a negative reaction for CO_2 (detected by seeing no bubbles), the soil rest was washed by 0.05 M HCl. After a negative reaction for Ca^{2+} (detected by ammonium oxalate), the soil rest was washed with distilled water. After a negative reaction for Cl^- (detected by $AgNO_3$), the soil rest was shaken in 0.1 M NaOH for 7–8 hours. The solution was precipitated overnight and centrifuged for 15 minutes at 5,000 rpm. A dark-brown HS mixture was precipitated by concentrated HCl to pH = 1. Coagulated HAs were decanted, washed several times, extensively purified by 0.5% mixture HCl + HF and dialyzed against distilled water until chloride-free, and freeze-dried (Pospíšilová et al., 2011 and 2016).

Diffuse reflectance infrared Fourier transform infrared (DRIFT) spectra were recorded over the range of 4,000–500 cm^{-1} in a homogenous mixture of 100 mg of HA samples and 300 mg dried KBr (FTIR grade 99%, Aldrich,

Germany) without any pressure. Mineral soil samples were observed by infrared spectroscopy as well. The soil samples were sieved through a 2 mm sieve and air-dried. For FTIR analysis, the sample (300 mg) was mixed with 900 mg KBr (FTIR grade 99%, Aldrich, Germany) and ground in an agate mortar. All spectra were measured using Thermo Nicolet Avatar 320 FT-IR spectrometer (Nicolet, Madison, WI, USA) operating with Smart Diffuse Reflectance accessory. Three FTIR spectra (absorption mode, KBr background, 256 scans, data spacing 1.929 cm^{-1}) were collected with each HAs and soil sample. The FTIR spectra were analysed at four absorption bands that indicate the aliphatic hydrophobic CH-groups (denoted as A) and aromatic COO-, C=C groups, and hydrophilic CO-groups (denoted as B, C, and D) (Demyan et al., 2012). C-H bands occurred at 3,000–2,800 cm^{-1} signal area and COO-, C=C and C-O bands occurred at 1,660–1,580, 1,546–1,520 and 1,740–1,600 cm^{-1} signal area). Spectra were integrated by the spectrometer software (Omnic, version 6a). Humification degree (B + C)/A and hydrophobicity index (D/A) were calculated (Ellerbrock et al., 2005; Margenot et al., 2015). The data were processed by analysis of variance followed by the Tukey HSD test that evaluates the significance of differences between the variants.

3   Results and discussion

Studied soils with varying degrees of degradation contain approximately 11% of carbonates and had a slightly alkaline exchangeable soil reaction (pH/KCl = 7.5). Average Cox content reached 1.47% on the control site, 1.31% on the accumulated site, and 0.94% on the erosion site. Humic substances content was as follows: 0.43% on the control site (= without signs of erosion-accumulation processes), 0.30 on the erosion site, and 0.36% on the accumulated site. The content of HAs varied from 0.15% on the erosion site to 0.20% on both control and accumulated sites. The quality of humic substances given by the humic and fulvic acids (HAs/FAs) ratio was the

highest on the control site and decreases in order: control (1.35), accumulation (1.23), and erosion (0.88). Similarly, Su Zheng-An et al. (2010) and Šarapatka et al. (2018) documented that erosion processes caused the transport of organic and inorganic materials to the concave part of the hill and the decrease of Cox on the eroded sites. Lack of organic matter according to Pospisilova et al. (2011) and Merlo et al. (2022) has a negative effect on soil biota and microbial activity.

Soil organic matter quality was assessed using DRIFT spectroscopy. For this reason, three HAs samples were isolated from soils with varying degrees of degradation. Figures 1 and 2 show that FTIR spectra of all studied samples suggested stretch vibration and deformation of the following functional groups: carboxylic and amido groups at 1,655–54 cm^{-1}; C=O bands at 1,690–1,716 cm^{-1}; carbonyl and carboxylic groups at 1,719–1,718 cm^{-1}; –C–H, CH$_2$; and CH$_3$ aliphatic groups at 2,942–2,920 cm^{-1}; amino- and amido-groups -N-H at 3,500–3,200 cm^{-1}. As it is evident from Table 1, studied HA samples varied in the amount of labile aliphatic hydrophobic groups (marked as column A, integrated area 3,000–2,800 cm^{-1}), aromatic stable and resistant C=C groups (marked as column B, integrated area 1,660–1,580 cm^{-1}), aromatic stable groups (marked as column C, integrated 1,546–1,520 cm^{-1}), and hydrophilic amido-, carboxylic-, keto-groups (marked as column D, integrated area 1,740–1,600 cm^{-1}). Humic acids on the accumulation site contained much more stable resistant aromatic groups and aliphatic groups to compare with erosion and control sites (columns A–D). The hydrophobicity index and decomposition/humification degree (index (B + C)/A) was also the highest in HAs on the accumulation site. We concluded that the erosion/accumulation process was the main factor affecting HAs quality on this site. On the erosion site, there were HAs more labile (column D), with a lower amount of stable aromatic groups (column B). Furthermore, higher values of decomposition/humification and lower hydrophobicity indicated a higher intensity of

Table 1   DRFIT spectroscopy parameters of studied HA and mineral soil samples and calculated indexes of decomposition/humification (index (B+C)/A) and hydrophobicity index (A/D)

Samples	A	B	C	D	Index (B+C)/A	Index A/D
HA–control	3.669b*	0.514NS	0.062NS	2.369a	0.157NS	1.561NS
Ha–erosion	2.628a	0.499	0.097	4.099b	0.228	0.647
Ha–accumulation	4.307c	0.951	0.102	2.497ab	0.25	2.053
Mineral soil – control	2.189b	2.715ab	0.077NS	5.542b	1.292b	0.396b
Mineral soil – erosion	4.492c	2.366a	0.071	4.360a	0.544a	1.032c
Mineral soil – accumulation	1.350a	3.066b	0.049	6.278c	2.313c	0.215a

*means within the column followed the same letter do not differ significantly as determined by Tukey multiple range tests (*P* <0.05); NS – non-significant differences; column A – integrated area 3,000–2,800 cm^{-1}; column B – integrated area 1,660–1,580 cm^{-1}; column C – integrated 1,546–1,520 cm^{-1}; column D, integrated area 1,740–1,600 cm^{-1}
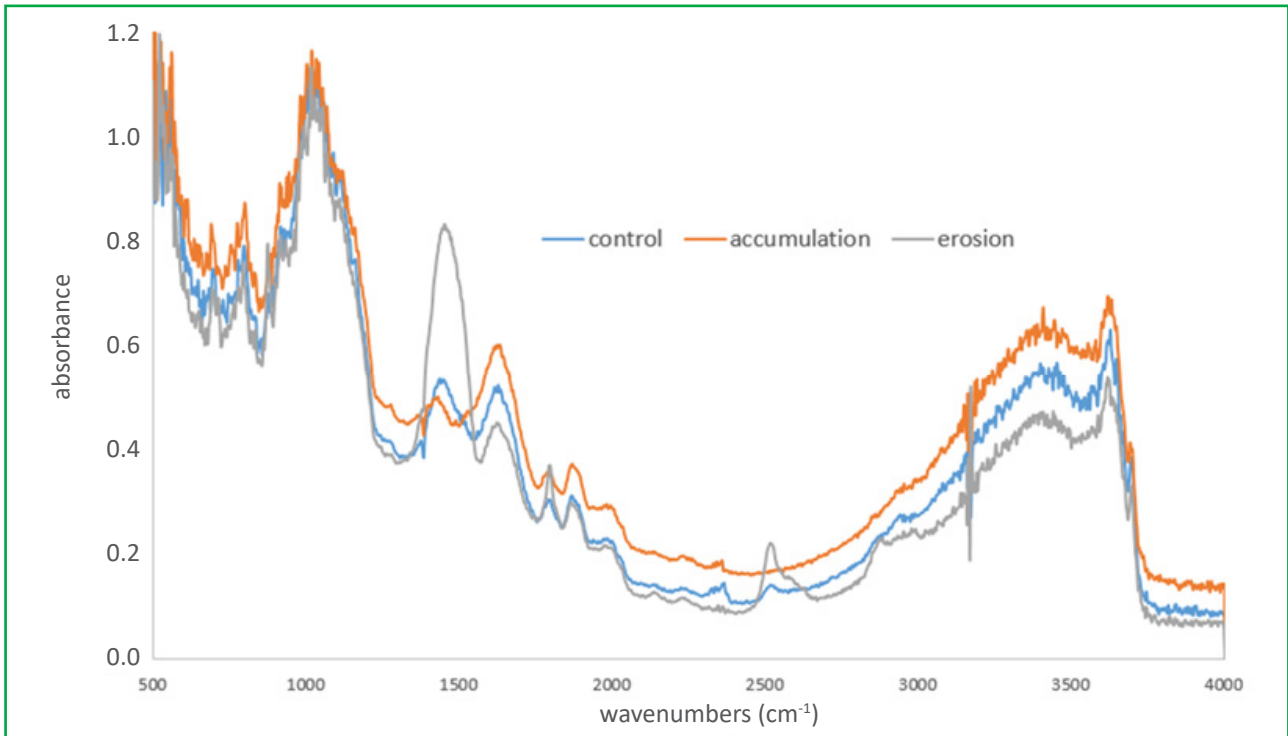
Figure 1   DRIFT spectra of Calcic Chernozem sampled along the transect C – control site, E – erosion site; A – accumulation site
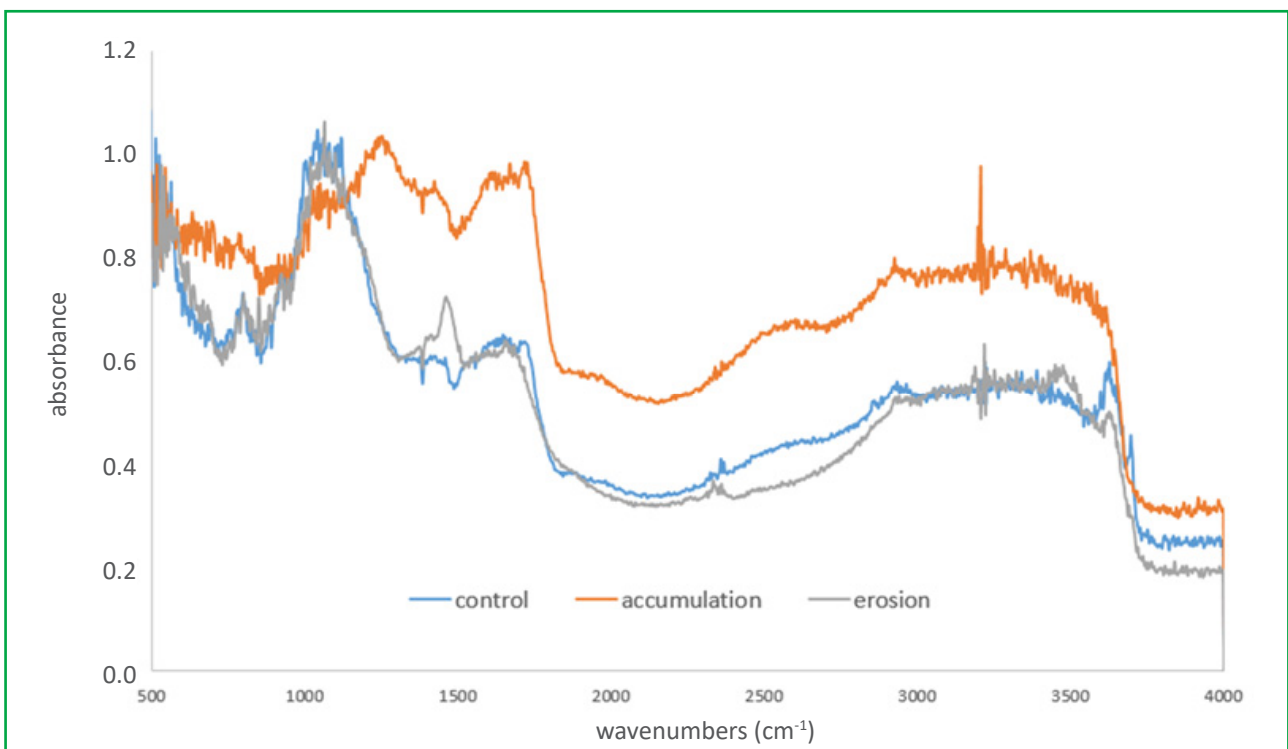

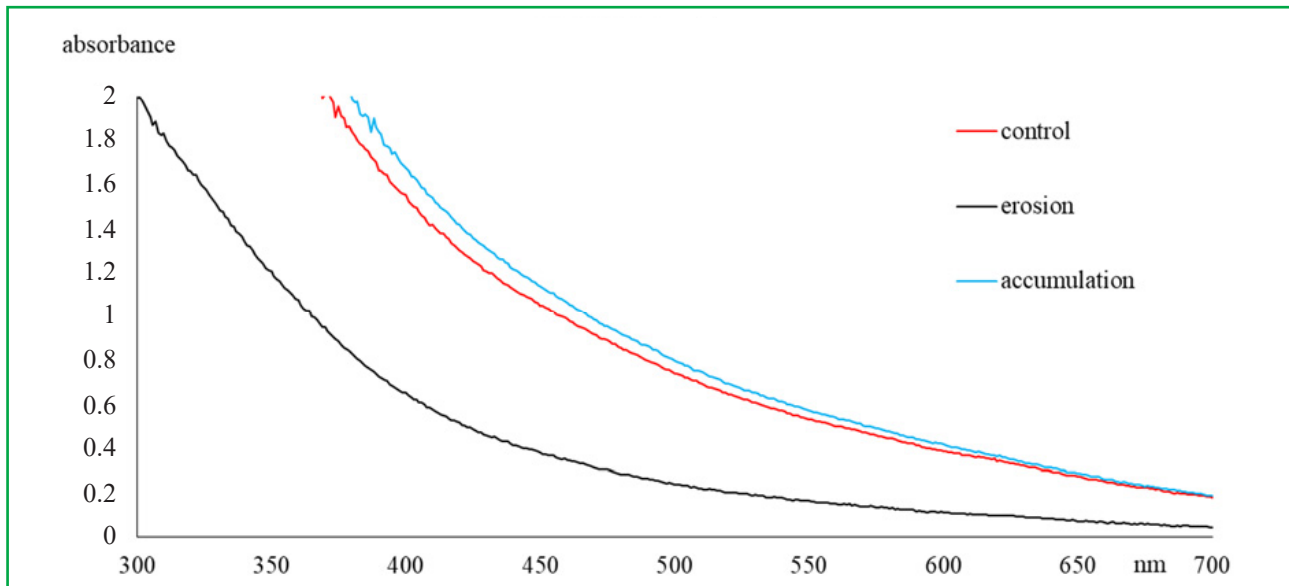**Figure 2**   DRIFT spectra of humic acids isolated from Calcic Chernozem

Figure 3   UV-VIS spectra of humic substances isolated from soil as affected by the erosion process

mineralization and microbial stress. Coates et al. (2000) demonstrated that amount of hydrophilic and aromatic groups and hydrophobicity of HAs can have a significant effect on the molecular morphology and binding constants of HAs for heavy metals and hydrocarbons and also directly affects the bioavailability of these compounds in the environment.

The presence of individual chemical components was also studied in the mineral soil samples. Generally, in the topsoil (= mineral soil), there was observed higher content of aliphatic labile and hydrophilic groups (see Table 1, columns A and D) on the erosion site to compare with control and accumulation sites. Content of aromatic stable and resistant components (Table 1, column B) was higher on both control and accumulated sites to compare with erosion site. It was concluded that this was caused by microbial stress and high microbial activity. The most stable and resistant aromatic components (column B) and hydrophilic groups (column D) are presented on the accumulation site because of erosion/accumulation process. The stability of organic components in the mineral soil samples varied and was also expressed by mutual ratios of absorbance and calculated indexes (see Table 1). The index (B + C)/A characterized the decomposition/humification degree. Lower values and microbial stress were typical for erosion site to compare with control and accumulation sites. Hydrophobicity (index A/D) on erosion was high and confirmed lower aggregability, wettability and HA quality because of soil degradation.

Our study demonstrate that the amount of aliphatic and aromatic compounds is affected by agro-technical measures, intensity of land use and erosion processes.

Because of lack of aliphatic C–H units, which control the water affinity, aggregates' stability, rate of the wetting, and adsorption processes became Calcaric Regosol more degraded and deteriorated (Ellerbrock et al., 2005; Šimon, 2008; Demyan et al., 2012). In addition, C–H units are responsible for, and contribute to the organic carbon cycle in the ecosystem (Demyan et al., 2012). Therefore, the lack of hydrophilic constituents in humic substances has also a negative effect on soil biota and microbial activity (Pospisilova et al., 2011; Merlo et al., 2022).

UV-VIS spectroscopy (Figure 3) proved the different humic substances' quality as affected by erosion/accumulation process along the studied field transect in Calcic Chernozem (control site), Calcaric Regosol (erosion site), and Calcic Chernozem Colluvic (accumulation site). The highest absorbance in UV-VIS spectral range was observed on the accumulation site, which corresponded with results of fractional composition and the highest humic substances content. Higher absorbance means higher humic substances quality. Calculated colour index Q4/6 was <4 on both accumulation and control sites. Values lower than 4 indicate high quality and stability of humic substances. On the other hand, higher Q4/6 values (4.2) indicated decreasing of humic substances quality on the erosion site and soil degradation.

4   Conclusions

Our results indicate that to analyse spectral properties only mineral soil is not enough to assess soil organic matter quality. It is recommended based on the research HAs isolation. The isolation is essential because of overlapping of absorption regions for mineral and organic soil component. Non-destructive DRIFT and UV-VIS

spectroscopic methods represent fast, and easy means of soil organic matter quality determination compared with labour and tedious classical humus fractionation methods. The assessment of soil organic matter quality is important, and it is frequently a part of various carbon cycle models. The high sensitivity of simple spectroscopic approaches provides detailed information of the presence of labile aliphatic and aromatic groups in humic substances. Calculated indexes of organic matter stability and hydrophobicity document clearly the aberration and degradation processes in intensively used Chernozems.

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