Comparison of soil organic matter composition under different land uses by DRIFT spectroscopy

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Abstract: The study aimed to estimate and characterise soil organic matter under different land uses (cropland, grassland, and forest) and soil depths. The soil organic matter composition of the soil was assessed by diffuse reflectance infrared spectroscopy (DRIFT). Humic and fulvic acids (HAs, FAs) were extracted from soils and their compositions were evaluated by DRIFT. Low molecular mass organic acids content was also measured. Our result revealed that the largest differences of the spectra in the composition of organic matter were observed in the upper parts of the soil profile. The forest soil spectra had more intense aliphatic bands, carboxylic, and CH bands than spectra of grassland and cropland soils. The difference of HAs spectra was at 3 010 to 2 800/cm where the most intensive aliphatic bands were in forest soil HAs, followed by grassland and cropland soil HAs. The grassland topsoil FAs spectrum differs most from the other land uses. It has lower peaks around 1 660–1 600/cm and 1 200/cm than cropland and forest. The concentration of low molecular mass organic acid (LMMOA) was the highest in the forest soil and the most abundant acid was citrate.

Keywords: terrestrial ecosystem; Luvisols; humus; organic compounds; functional groups

Soil organic matter (SOM) plays an important role in biological, chemical, and physical soil improvement and productivity (Strosser 2010). The living biomass including microorganisms breaks down the plant residues or detritus and animal waste into humus or organic matter by using carbon as an energy source and nitrogen as a source of protein production (Allison et al. 2007). The decomposition of plant residues releases the organic chemical compounds and helps to cling together with the mineral soil particles that improve the chemical soil properties by soil sorption complex creating and physical soil properties by establishing of soil structure (Davidson and Ackerman 1993).

However, SOM in the ecosystem has been stored in different layers with different concentrations as a result of different stages of decomposition (Ribeiro et al. 2001). The quantity and the quality of SOM depend on several factors such as duration of decomposition, residues, roots, amount of fine materials, type of decomposers (microorganisms), chemical composition, and temperature (Lal 2018). On the other hand, the fluctuation of the organic matter concentration in the soil is related intensively to slopes, elevation, topography, soil types, and land uses and management (Slepetiene and Slepetys 2005, Jakšík 2015). The humic substances such as humic acids (HAs) and fulvic acids (FAs) are also the component used to identify the quality of SOM. They play an important role in the terrestrial ecosystem (Trevisan et al. 2010), and they are known as a mixture of substances in the form of supramolecular

structures (Piccolo 2001). Humic substances make up about 20% of the total of SOM and result from the decomposition and humification process of the SOM (Pavlů and Mühlhanselová 2017). They are often understood as relatively stable components of SOM, which are involved in the fixation and sequestration of carbon in the soil (Lal 2005). The low molecular mass organic acid (LMMOA), which makes up about 10% of dissolved organic carbon (DOC), also characterises the compositions of soil organic matter. They are carboxylic acids of low molecular weight (Ash et al. 2016) and could be aromatic or aliphatic (Hubová et al. 2017). LMMOA are understood as relatively variable and unstable components of SOM (Strobel 2001).

Diffuse reflectance infrared fourier transform spectroscopy (DRIFT), as one of the types of infrared spectroscopy conventionally used for solid powder samples, is commonly used to analyse peat soil, composts, and the transformation of organic matter during composting within various stages (Haberhauer and Gerzabek 1999, Zaccheo et al. 2002, Pavlů and Mühlhanselová 2017). The DRIFT spectra have been recognised as one of the spectroscopic techniques used to distinguish the fluctuation in the abundance of organic functional groups during decomposition and to identify the changes of SOM in the soil profile under different vegetation covers (Veum et al. 2014).

The study hypothesised that different land uses are connected with different incoming fresh organic materials and these differences can be seen throughout the whole soil in the composition of organic matter. Therefore, the study aimed to describe and compare the SOM compositions and their transformation under different depths and vegetation covers. The comparison of the separated organic compounds such as humic and fulvic acid and low molecular mass organic acid was observed by the combination of the advanced analytical methods.

MATERIAL AND METHODS

Site selection and soil sampling. The research was conducted on the outskirts of Prague, Suchdol (Czech Republic). The area is situated in altitude range 250–300 m a.s.l. and has a mean annual precipitation of about 470 mm and a mean average temperature of 11 °C. The mixture of loess and sandy river sediments of the Quaternary age creates the bedrock of the research area. Haplic Luvisols are the prevailing soil type in all land uses. Cropland site (with

a dominantly grown wheat (*Triticum aestivum* L.) crop interspersed with rape (*Brassica napus* L.) and maize (*Zea mays* L.)) marginally include the areas of greyic Phaeozems and carbonates were detected in several soil samples mainly in deeper layers. Soil texture belongs to clay loam category. Same soil description applies to grassland site (poorly maintained grassland with *Dactylis polygama* Horv., *Poa annua* L., *Calamagrostis epigejos* Roth). Broadleaf forest site with the dominant abundance of oak (*Quercus petraea* (Matt.) Liebl.) followed with beech (*Fagus sylvatica* L.) and hornbeam (*Carpinus betulus* L.) marginally include the areas of Regosols and Cambisols. The presence of carbonate was not detected in all soil samples from the forest. Soil texture belongs to sandy clay loam category.

Ninety soil samples were collected from each land uses and categorised for the three different depths (0–10 cm (with exclusion of litter layer in forest), 10–20 cm and 20–30 cm). In all cases, the samples from the first two layers captured humic (A) horizon. Samples from the deepest layer captured either still horizon A (in the case of Phaeozems or its gradual transformation to eluvial horizon in case of Luvisols, to cambic horizon in Cambisols or to mineral substrate in Regosols.

The taken samples were air-dried and sieved with a 2 mm sieve. Furthermore, 2-mm-sieved soil samples were milled (Fritsch Analysette 3 Spartan Pulversette miller, Idar-Oberstein, Germany) into very fine particles to use for infrared spectroscopy. Fulvic and humic acid were extracted from selected 18 soil samples. The fresh topsoil (15 soil samples) was taken separately and frozen for analysing the DOC and LMMOA.

Soil analysis. The exchangeable (pH_{KCl}) was determined potentiometrically by the pH-electrode SenTix 21 (Inolab pH level 21, WTW, Prague, Czech Republic). Soil organic carbon (SOC) was measured by using rapid dichromate oxidation techniques (Sparks 1996). The quality of humus was determined by the absorbance ratio of sodium pyrophosphate ($Na_4P_2O_7$) soil extract at 400 nm and 600 nm (E4/E6, respectively) (Sparks 1996). The content of LMMOA was measured using ion chromatography (IC) with suppressed conductivity (Hubová et al. 2017). Dissolved organic carbon content was measured by the wet dichromate oxidation method according to Tejnecký et al. (2014).

The extraction of humic substances was carried out by the international humic substance society (IHSS)

Table 1. Describing the soil characteristic among the different depths (0–10, 10–20 and 20–30 cm)

	Depth (cm)	pH_{KCl}	Humus quality index	Soil organic carbon (%)
Cropland	0–10	6.79 ± 0.37	3.27 ± 0.37	1.37 ± 0.17[a]
	10–20	6.76 ± 0.33	3.25 ± 0.34	1.41 ± 0.20[a]
	20–30	6.85 ± 0.43	3.24 ± 0.43	1.11 ± 0.13[b]
P-value		0.852	0.977	0.001
Grassland	0–10	5.93 ± 0.19	3.50 ± 0.36	2.11 ± 0.31[a]
	10–20	5.73 ± 0.79	3.30 ± 0.17	1.58 ± 0.25[b]
	20–30	5.91 ± 0.70	3.28 ± 0.20	1.51 ± 0.38[b]
P-value		0.788	0.149	0.000
Forest	0–10	3.59 ± 0.21	4.41 ± 0.43	5.64 ± 2.54[a]
	10–20	3.52 ± 0.13	4.56 ± 0.42	1.78 ± 0.49[b]
	20–30	3.65 ± 0.10	5.02 ± 1.17	1.06 ± 0.34[b]
P-value		0.206	0.195	0.000

Data (means ± standard deviation; *n* = 10)

fraction method, which is modified by Piccolo et al. (2000). A mixture of NaOH and $Na_4P_2O_7$ was used to extract the humic substances. The extract was acidified to pH 1.0 using HCl for precipitation of humic acids and their separation from fulvic acids. The HAs fractions were purified by redissolution with NaOH and reprecipitation with HCl. The purification from co-extracted clay was completed with the solution of HCl and of HF. The suspension was neutralised, centrifuged, and dialysed to release chlorine and then the HAs were freeze-dried. The FAs solutions were purified using the hydrophobic resin in the column. The FAs were released from the sorption of resin using NaOH solution. Finally, the FAs were neutralised, dialysed, and freeze-dried.

DRIFT spectra of pure freeze-dried humic acids, fulvic acid, and dried fine soil samples were recorded by the infrared spectrometer (Nicolet iS10, Waltham, USA). The spectra with a range of 2.5 to 25 μm (4 000 to 400/cm) were used. The gold mirror was used as a background reference. The 64 scans with resolution 4/cm and Kubelka-Munk units were applied. OMNIC 9.2.41 software (Thermo Fisher Scientific Inc., Waltham, USA) was applied for spectra analysis.

Data analysis method. The software IBM SPSS (version 26, New York, USA) was used for data analysing and One-way ANOVA was applied for determining the statistical differences among quantitative soil characteristics with different land uses, and depths at significance level description $P < 0.05$. Tukey test and letters a, b, c were used to describe the significant differences, where a is the highest value, followed by b and c.

RESULT AND DISCUSSION

Basic soil characteristics. The analysed data (Tables 1 and 2) indicated that there are no significant differences for the pH_{KCl} among all three depths in all land uses. However, the soil in crop-

Table 2. The differences description of basic soil characteristics among the three land uses (cropland, grassland, and forest)

	pH_{KCl}			E4/E6			Soil organic carbon		
	0–10	10–20	20–30	0–10	10–20	20–30	0–10	10–20	20–30
					(cm)				
Cropland	a	a	a	b	b	b	b	ns	b
Grassland	b	b	b	b	b	b	b	ns	b
Forest	c	c	c	a	a	a	a	ns	a
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.000

Letters indicate significant difference; ns – none significance; E4/E6 – the humus quality index

land is neutral, in grassland is moderately acidic while in forest is strongly acid. Similarly, the method used for the indicative evaluation of SOM quality (E4/E6) in different depths in all the land uses had no significant differences. The cropland and grassland have very good humus quality while the forest does not. The higher E4/E6 ratio in forest could indicate lower degree of humification processes (Kunlanit et al. 2019). Soil organic carbon content is significantly different among the three depths of all land uses. Table 2 shows in the depth 0–10 cm, the SOC has the highest content in the forest, followed by the grassland and cropland. For the 20–30 cm depth, the grassland has the highest SOC comparing to SOC in the cropland and forest. Various studies found the same result, that grassland had higher SOC than cropland and forest in deeper soil layers (Muktar et al. 2018).

DRIFT spectra

Spectra of soils. Position and identification of soil spectra bands are presented in Table 3. The spectra of the cropland soil are very similar in all sampled depths (Figure 1). This corresponds well to tillage and soil stirring. In the soil spectra of the different depths under the grassland, there are also no differences in bands position, intensities, or shapes. The forest soil spectra of 0–10 cm layer differ from the

two deeper layers. The higher content of aliphatic components is evident from band absorbance in the range between 3 000–2 800/cm.

The most obvious differences among land uses are visible in soil spectra of the surface layer. The spectrum of forest soil differs from others. The bands of aliphatic groups are well identifiable between 3 000 and 2 800/cm. The extension of the band with a maximum from around 1 660/cm to the region of the carboxyl group (1 720/cm) is apparent and the band shape differs between 1 500 and 1 200/cm (polyphenolic substances and functional groups with nitrogen and phosphorus). The band around 920/cm documents a lower content of secondary alumosilicates (apparent in the whole profile), which corresponds to more sandy soil texture in forest. Hence, a large proportion of aliphatic, carboxylic, aromatic, and CH groups under forest correspond with higher organic carbon content in this soil (Gerzabek et al. 2006).

In the deeper layers of forest soil, the shoulder of carboxyl groups in the band with a maximum around 1 640/cm is still visible. The dominant peak of the forest soil spectrum is the band around 1 300/cm. In the depth 20–30 cm, the band at 1 040–945/cm (indicating C-O stretching, Si-OH of alumino-silicate lattice, and carbohydrate region of polysaccharides) is lower under forest than grassland and cropland. The polysaccharides content decrease through the

Table 3. The assignment of the major bands in infrared spectra of the soil (Tinti et al. 2015, Matamala et al. 2017)

| Wavenumber (1/cm)	Assignment of sorption bands
3 600–3 700	Si-O-H vibration of clays
3 440–3 320	O-H and N-H stretching, H-bonded OH
3 010–2 800	aliphatic CH stretching
2 000–1 790	Si-O vibration of quartz mineral
1 775–1 711	C=O stretching in carboxylic group
1 691–1 642	C=O stretching of amides (amide I), H-bonded conjugated ketones, carboxyls and quinones, lignin, C=N stretching
1 642–1 569	amide II of primary amides, aromatic C=C, C=O (quinones), carboxylates
1 544–1 488	aromatic C=C stretching, aromatic skeletal vibration, aromatic (lignin), amide II
1 479–1 444	CH and NH of amide II, aliphatic CH deformation, carbonates
1 444–1 408	C-H deformation and C-O stretching of phenolic groups
1 403–1 354	C-O of phenolic OH, COO$^-$ and O-H, CH$_3$ bending,
1 342–1 307	C-N (aromatic amines)
1 293–1 256	C-O of aryl ethers, C-O of phenols, C-O-C ether bond, bentonite
1 256–1 198	C-O stretching and OH deformation of COOH, C-O of aryl ethers and phenols, silicate
1 185–1 070	C-OH of aliphatic alcohols, O-Si-O stretching of quartz, sulfates
1 056–945	C-O stretching, polysaccharides, Si-OH of alumino-silicate lattice (kaolinite, illite)
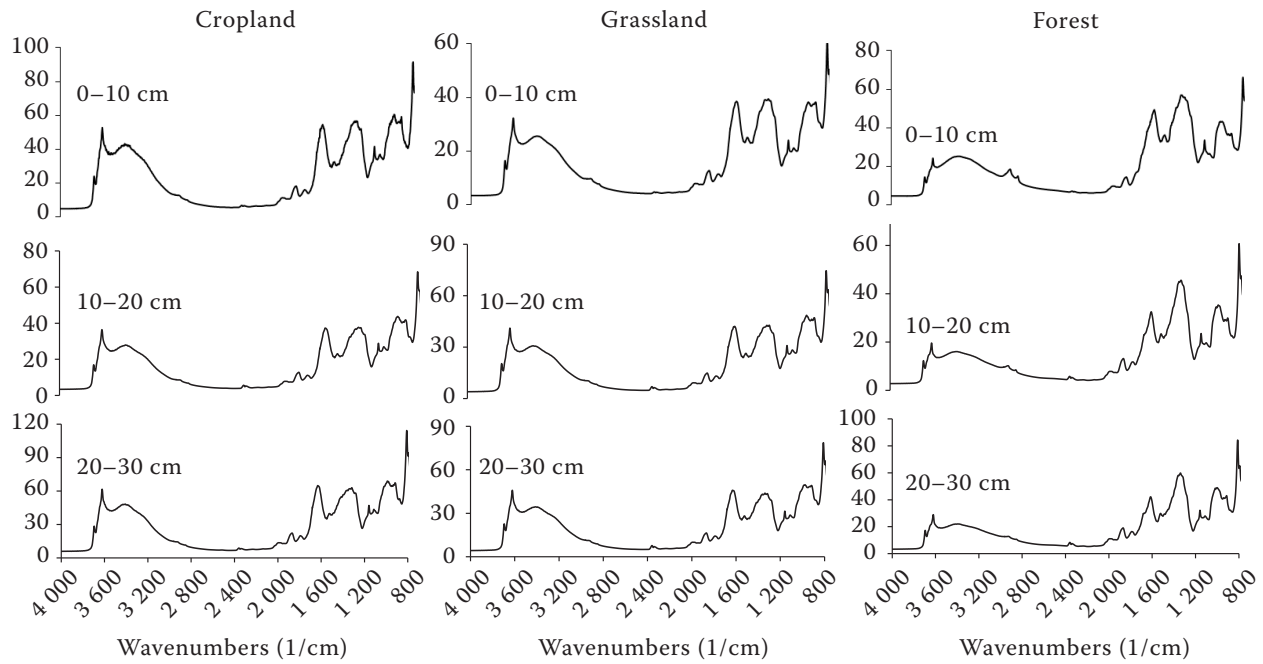
Figure 1. The difference of average soil spectra under different depths (0–10, 10–20 and 20–30 cm) and land uses (cropland, grassland, and forest)

depth of the forest soils was documented by Sugiura et al. (2017) and probable a higher appearance of inorganic materials (Haberhauer et al. 1998).

Spectra of humic acids. The main bands of HAs and FAs spectra are described in Table 4. The dominant peak of these spectra is a peak around 1 740/cm,
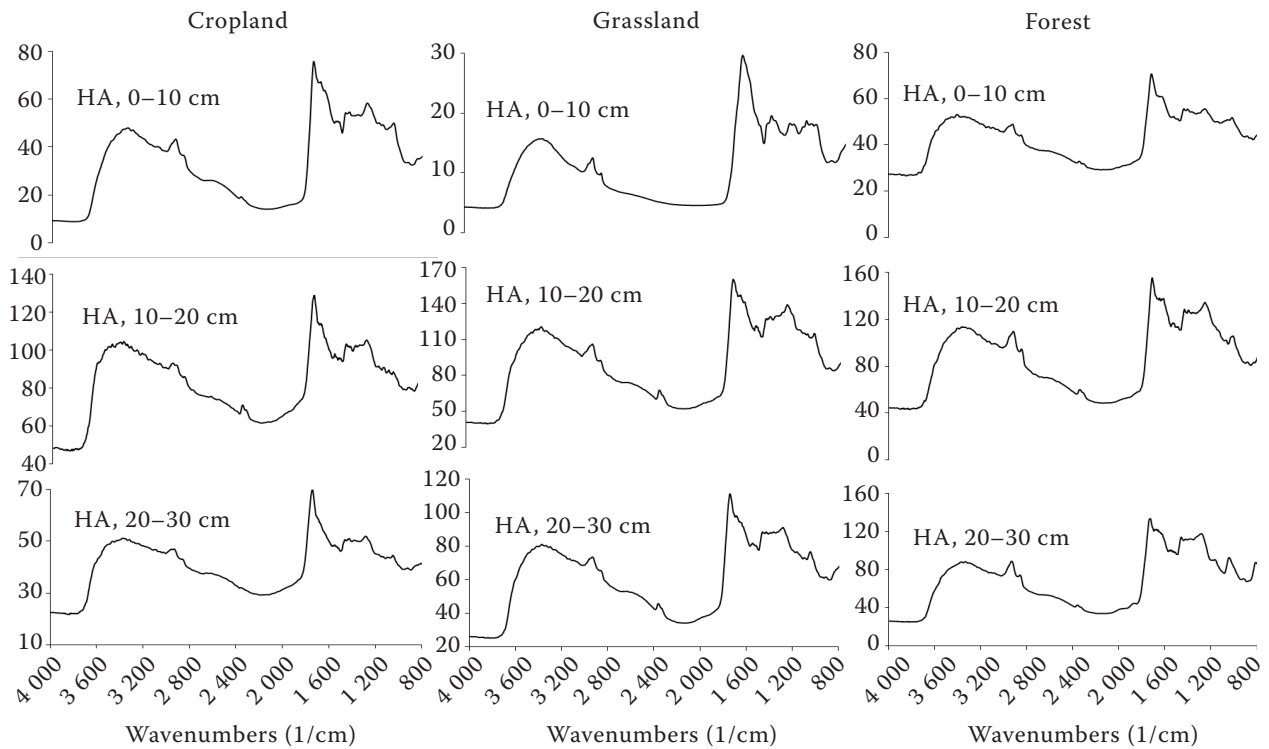

Figure 2. The difference of average humic acids spectra under different depths (0–10, 10–20 and 20–30 cm) and land covers (cropland, grassland, and forest). HA – humic acid

Table 4. The major bands of humic substances (humic and fulvic acids) in infrared spectra (Stevenson 1995, Tatzber et al. 2007, Pavlů and Mühlhanselová 2017)

Wavenumber (1/cm)	Assignment of sorption bands
3 400–3 300	O-H stretching, N-H stretching
2 950–2 800	aliphatic C-H stretching
1 725–1 710	C=O stretching of COOH and ketones
1 660–1 630	C=O stretching of amide I, quinone, H-bonded conjugated ketones
1 620–1 600	aromatic C=C stretching
1 590–1 517	N-H bending and C=N stretching (amide II)
1 470–1 380	aliphatic C-H bending
1 400–1 390	OH deformation of CH_2 and CH_3
1 280–1 200	C-O stretching and OH deformation of COOH, C-O stretching of aryl esters
1 170–950	C-O stretching of polysaccharides

which represents the carboxylic groups on aromatic rings (Figure 2). The vibration band of the carboxylic group is typically placed near 1 720/cm in the case of substitution on aliphatic chains. In case of substitution on aromatic rings is placed just near 1 740/cm (Reddy et al. 2018).

The surface layer of the cropland soil differs from deeper layers, more pronounced peak can be seen around 3 000–2 800/cm and 1 000/cm, which represents higher contents of aliphatic components and polysaccharides chains of HAs. It could point to their lower maturity and stability (Pavlů and Mühlhanselová 2017). The intensity of the C=O group of ketones and amide group (shoulder in range 1 690–1 630/cm) decreases with soil depth.

The spectrum of the surface layer of the grassland varies by the spectra from other depths and also from other land use. The band around 1 660/cm is dominant, while the carboxyl band is hidden in the spectrum, and the band around 1 280/cm is relatively less pronounced in comparison to other spectra. The aliphatic-bending at 1 460/cm is shifted to 1 425/cm in grassland HAs spectra of the surface soil layer, while in forest and cropland HAs spectra are clearly visible at both positions. It might be the formation of H-bonds between hydroxyl and carboxyl H atoms of HA (Senesi et al. 2001).

HAs spectra of deeper layers of forest soil, differ from others in pronounced aliphatic bands (3 000–2 800/cm). In addition, it could be connected with more sandy substrate in forest as described by Di et al. (2016). The forest HAs spectra have relatively (compared with neighboring band around 1 720/cm)
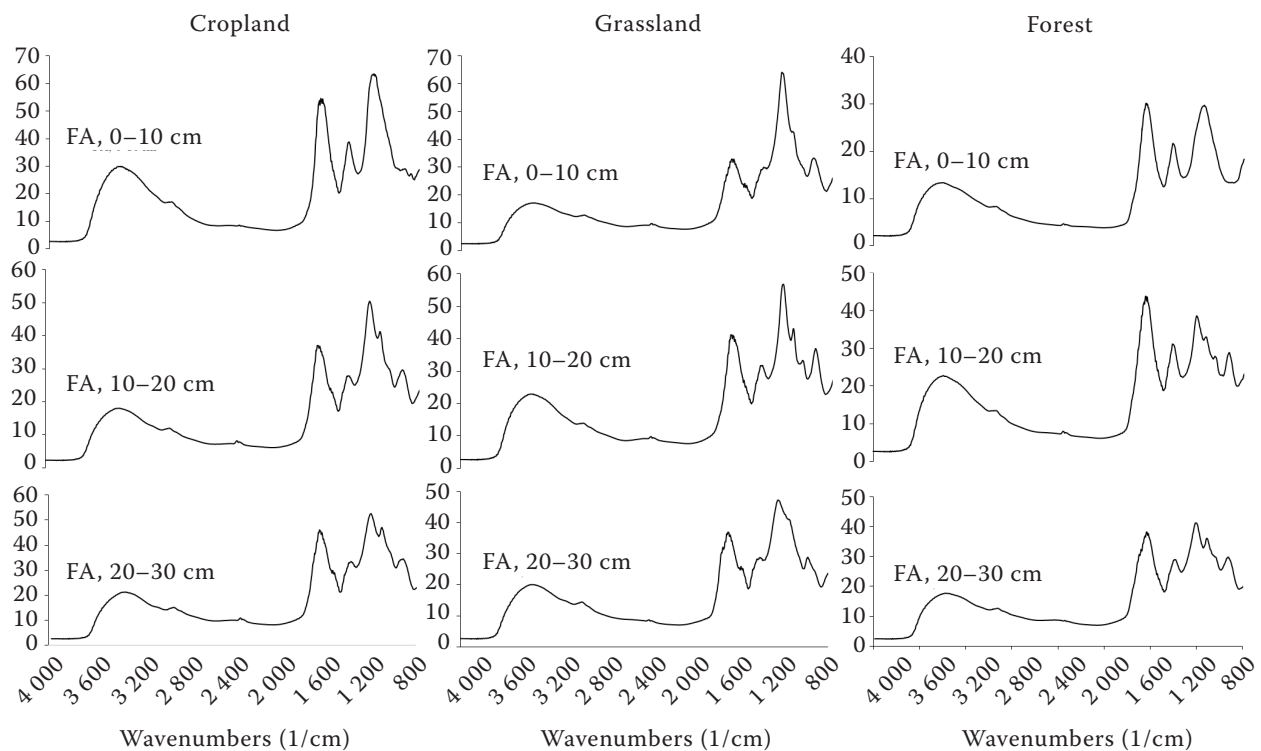

Figure 3. The difference of average fulvic acids spectra under different depths (0–10, 10–20 and 20–30 cm) and land uses (cropland, grassland, and forest). FA – fulvic acid
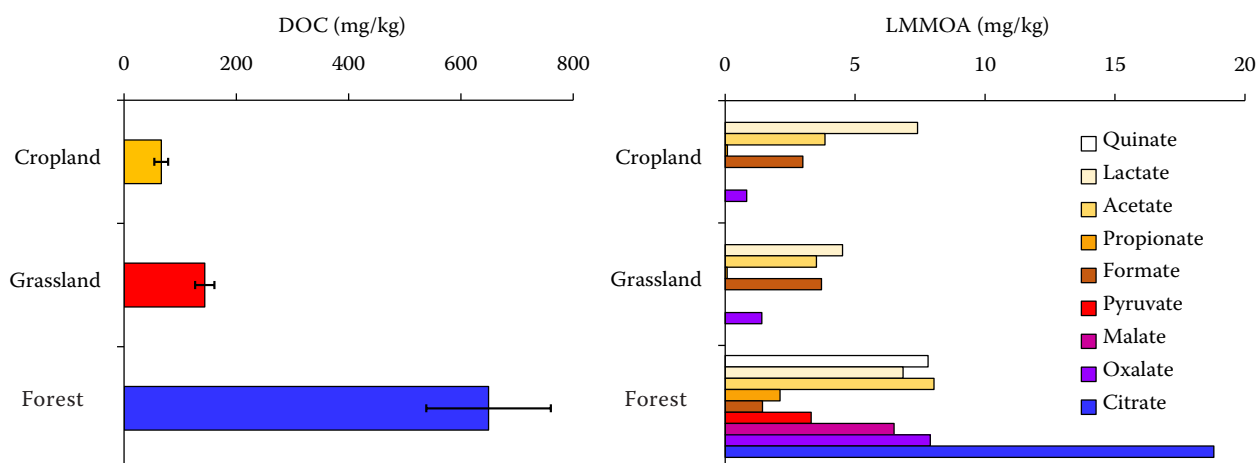
Figure 4. The mean concentrations of dissolved organic carbon (DOC) (error bars show standard deviations) and low molecular mass organic acid (LMMOA) under different land uses (*n* = 5)

the highest peak around 1 660/cm in comparison with other land use and soil depth below 10 cm. The band of aliphatic C-H (1 470–1 460/cm) is also higher under forest than the grassland and cropland.

Spectra of fulvic acids. Generally, the spectra of FAs have a lower amount of peaks in the fingerprint area (Figure 3). More details are visible in the spectra of lower parts of the soil profile, where bands of polysaccharide chains and deformation vibrations of OH groups in carboxyl appear.

The FAs spectra from different soil depths under cropland are quite different. The band at 1 670–1 600/cm, which mainly characterises carboxyl, ketones, and aromatics, is clearly visible in all three depths. However, the shoulder at 1 570–1 560/cm (COO- symmetric stretching, N-H deformation,

and amides group II) is more pronounced in the deeper layers. The band at 1 420–1 400/cm (phenols and alcohols) is sharp in the uppermost layer. The spectra of FAs from surface layer of grassland have a bigger amount of peaks in the fingerprint area. The relative intensity of the band 1 680–1 630/cm and 1 100–1 200/cm increase with depth. The grassland FAs spectrum differs most from the other land uses in the depth of 0–10 cm. It has significantly lower peaks around 1 660–1 600/cm and 1 200/cm than cropland and forest. The band at 1 560–1 510/cm is more intense under grassland than cropland and forest. Gerzabek et al. (2006) found that aromatic and NH groups were greater in grassland than arable land. The FAs spectra of the surface forest soil layer have only three wide peaks in the fingerprint area. In the

Table 5. The description of low molecular mass organic acid (LMMOA) and dissolved organic carbon (DOC) concentration under different land uses in the upper layer (0–10 cm); means ± standard deviation; *n* = 5

		Cropland	Grassland	Forest
LMMOA (mg/kg)	quinate	bdl	bdl	7.81 ± 8.13
	lactate	7.40 ± 6.26	4.52 ± 1.35	6.85 ± 1.57
	acetate	3.84 ± 2.34	3.51 ± 3.57	8.04 ± 11.89
	propionate	0.09 ± 0.05	0.08 ± 0.08	2.11 ± 1.67
	formate	2.99 ± 2.65	3.71 ± 3.89	1.44 ± 0.88
	pyruvate	bdl	bdl	3.31 ± 2.95
	malate	bdl	bdl	6.50 ± 5.77
	oxalate	0.83 ± 0.52	1.41 ± 0.69	7.89 ± 6.63
	citrate	bdl	bdl	18.8 ± 25.08
DOC (mg/kg)		66 ± 27.36	144 ± 38.55	649 ± 247.92

bdl – below determination limit

FAs spectra of a deeper layer are visible their splitting on several peaks. The forest FAs spectrum under the depth 10–20 cm has the opposite intensities ratio of bands (1 660 ≥ 1 200) to the other two land uses (1 660 ≤ 1 200). It means that the forest has a higher presence of quinone, ketones, and aromatic C=O than C-O and OH deformation of COOH. It is in accordance with the work of Leinweber et al. (2001).

Dissolved organic carbon and low molecular mass of organic acids. On the base of previous results, the most differences among land use are focused on top parts of the soil profile where LMMOA was mostly found (Hubová et al. 2017). The description of this part of the profile is therefore extended to DOC and LMMOA evaluation (Figure 4, Table 5).

The concentration of DOC is relatively high under forest, followed by grassland and cropland. Lower DOC in cropland may result from ploughing, drainage, intensive surface runoff, which cause DOC losses (Manninen et al. 2018). Forest was found to have the highest concentration of LMMOA (citrate, acetate, quinate, oxalate, malate, pyruvate, propionate, formate) followed by grassland and cropland. Citrate concentration is higher under forest while lactate concentration is higher under grassland and cropland. Hubová et al. (2017) showed that more acidic soil contains a higher concentration of citrate. The big value of standard deviation in LMMOA is natural for this slightly stable and highly variable component of soil organic matter. On the other hand, it has a correlation between the amount of LMMOA and DOC with *P*-value 0.01 ($r = 0.755^{**}$) under all land uses. The high concentration and amount of LMMOA are based on plant root exudation, residues, and litters decomposition reviewed by (Adeleke et al. 2017, Hubova et al. 2017), and the highest content in forest is as a result of litter decomposition (Berg and McClaugherty 2020) and lower pH in forest area (Rukshana et al. 2014).

It can be summarised that land uses influence the amount and qualitative parameters of soil organic matter. Infrared spectroscopy is a useful tool for composition of the SOM evaluation. The most obvious differences in SOM composition according to land use are evident in surface layer of soil. Forest soil spectra had more intense aliphatic bands (3 010–2 800/cm) than the grassland and cropland in the upper layer. Similarly, the HAs spectra of forest soil have more intense aliphatic bands than the grassland and cropland HAs. More acid characters of organic matter in forest soil are also documented by soil spectra

in the intensity of carboxylic bands. The grassland FAs spectrum differs most from the other land uses in the depth of 0–10 cm. It has significantly lower peaks around 1 660–1 600/cm and 1 200/cm than cropland and forest. In the cropland soils, aromaticity of HAs increase with depth. The concentration of LMMOA was higher under the forest, followed by grassland and cropland. The most abundant acid in LMMOA mixture was citrate in the forest while lactate was in the grassland and cropland.

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