

Effect of Organic Fertilizers on Soil Organic Carbon and Risk Trace Elements Content in Soil under Permanent Grassland

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Abstract

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The effect of different kinds of organic matter inputs on elemental composition, content, and quality of humic substances was studied on permanent grassland during 2008–2013. The experiment included two organic fertilizer types – compost and slurry, both with the range of stocking rates 0.9, 1.4, and 2.0 livestock units (LU)/ha (corresponding to 54, 84, and 120 kg N/ha, respectively), and control without any fertilizer. The soil was sandy-loam, of Cambisol type, with semi natural permanent grassland. Labile forms and total contents of selected trace elements (Co, Cu, Zn, Cd) and macroelements (Ca, Mg, K, P, and N) were measured. Soil organic matter parameters such as total organic carbon (C_{ox}), humic substances (HS), humic acid (HA), fluvic acid (FA), and labile carbon forms (hot-water extractable carbon (C_{hws}), cold water extractable carbon (C_{cws})) were determined. Results showed that the greatest content of P, Ca, Mg, and N in the soil was detected by the CO 2.0 treatment. Furthermore, the positive effect of compost on C_{ox} , C_{hws} , HA, FA, HS, N and macronutrients was observed. The elemental analysis of humic acids showed lower carbon content and higher oxygen content in HA molecule, which indicated young humic acids, with a lower condensation degree. The content and quality of humic substances strongly influenced the total and labile trace elements content in the soil. Additionally, hot water soluble carbon significantly correlated with plant available forms of Zn, Cu, and Cd.

Keywords: compost; labile organic carbon; slurry; trace elements

Soil organic carbon is one of the most important soil components due to its ability to affect plant growth, being both an energy source and a trigger for nutrient availability through mineralization (EDWARDS *et al.* 1999). Stability or lability of any organic carbon fraction could be due to either chemical composition or protection within the soil aggregates. Stable organic carbon forms are characterized by redox properties and can act as electron acceptors or donors in natural environment. They can influence the oxidation state of metal ions and thus their speciation and mobility (PALMER *et al.* 2006; AESCHBACHER *et al.* 2010). Chemical forms, metal ion complexation by humic substances

and their influence on the content of mobile and potential mobile heavy metals in soil was also studied by SENESI and LOFFREDO (2005); GONDAR *et al.* (2006), and DERCOVA *et al.* (2007). Metals of anthropogenic origin were considered as more available in soils than those from parent rock. Most of metals in plants are taken up by roots from soil. Some heavy metals are accumulated in roots, leaves, stems, and fruits, while others are well mobile in plants. KABATA-PENDIAS (2004), HANČ *et al.* (2008), BIDAR *et al.* (2009), KOO *et al.* (2010), PADMAVATHIAMMA and LI (2010) studied phytoavailability of macro, micro and trace elements. HEJCMAN *et al.* (2010) described the relationship be-

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tween soil and biomass chemical properties, amount of elements applied, and their uptake on grassland. They claimed that there is no simple positive relationship between the applied elements and their concentrations in the plant biomass on grassland.

Labile dissolved organic carbon (DOC) is represented by a complex mixture of organic molecules of varied origin that occurs in soil solution. LEINWEBER *et al.* (1995) supposed that water-extractable organic carbon is the main component of labile organic carbon, and whether or not a particular organic molecule is dissolved in water depends on the water content, nature of the surfaces and other solutes. DOC is very active in carbon pool and its chemical composition and mobility (smaller and more polar organic molecule) directly affect biological soil properties because of providing energy and nutrients. ZSOLNAY (2003) defined dissolved organic matter (DOM) (with the possible exception of the colloids) in the hydrosphere by size limit (= through filtration). The size limit, which is used to differentiate DOM from particulate organic matter, is arbitrary, nevertheless there is an almost universal consensus that it is around 0.45 μm . Another potential problem of DOM definition is possible structural artefacts in DOM caused by abnormally high concentrations and drastic pH changes. Associations between DOC, heavy metals, and other hydrophobic pollutants were studied by ROOK (1974) and HAYES and CLAPP (2001). It was proved that fulvic acids, as the precursor of carcinogenic pollutants, are harmful to human health. GHANI *et al.* (2003) and UCHIDA *et al.* (2012) quoted that labile organic carbon is responding to changes in rhizosphere caused by management practices and is considered as an important indicator of soil quality/health. Many studies have reported about DOC changes and their dynamic, which is influenced by grazing, fertilization, mowing, and mulching (HAYNES 2000; VÁCHALOVÁ *et al.* 2013). MONTEITH *et al.* (2007) supposed that the content of DOC could increase in soil solution with a warmer climate,

changes in the amount of precipitation or decline in atmospheric deposition. DEBOSZ *et al.* (2002) found a significantly lower content of DOC in unfertilized soils compared to fertilized arable soils. For labile carbon estimation, the use of microbial biomass carbon was recommended by DEGENS and SPARLING (1996), while JANZEN *et al.* (1992) suggested using light-fraction organic carbon. Although there are no standard methods to isolate soil nanoparticles or nano size structures, the nano-scale dimension gains a considerable interest and appears to be useful for studying organic matter properties (THENG & YUAN 2008; MONREAL *et al.* 2010). ŠESTAUBEROVÁ and NOVÁK (2011) determined extractable soil carbon and DOC in peaty soils. They observed that not all dissolved carbon could be extracted from soil. The extraction is influenced mainly by the extraction agents, isolation procedure, and by pH. Therefore instead of complicated measuring DOC, the water extractable carbon is more frequently determined.

The objectives of the present study were: (1) to evaluate the impact of organic fertilizers on the content and quality of soil organic matter, (2) to determine the elements content in soil and in the humic acids molecule, (3) to elucidate the relationship between the humic substances and the elements content.

MATERIAL AND METHODS

Site description. The experimental plots were set up on permanent grassland in 2004. Plots were located in the northwest part of Moravia close to Rapotín (400 m a.s.l.). Annual average air temperature is 7.7°C and annual rainfall average is 693 mm. The experimental locality is characterized by semi-natural permanent grassland. Soil type was classified as Haplic Cambisol according to NĚMEČEK *et al.* (2001). The soil was sandy-loam textured and its basic characteristics are given in Table 1.

Experimental design. The treatments consisted in two systems of organic fertilizer application, slurry (SL)

Table 1. Basic soil characteristics of Haplic Cambisol

Horizon (cm)	pH/KCl	Conductivity (mS/cm)	C _{ox} (%)	HA/FA	Q4/6	Clay	Silt	Sand
						(%)		
Ad (0–8)	5.7	0.04	0.9	0.6	6	9.4	32.8	57.9
Ao (8–30)	5.7	0.04	0.8	0.6	7	–	–	–
Bv (30–50)	5.7	0.01	0.5	–	–	–	–	–
BC (> 50)	–	–	–	–	–	–	–	–

HA – humic acid; FA – fulvic acid; C_{ox} – total organic carbon

Table 2. Experimental design of plots

Treatment	Fertilisation	Livestock unit (LU/ha)	Amount of applied N (kg/ha)	Amount of cuts (cuts/year)
C	without (control)	0	0	2
SL 0.9	slurry	0.9	54	2
SL 1.4	slurry	1.4	84	3
SL 2.0	slurry	2.0	120	4
CO 0.9	compost	0.9	54	2
CO 1.4	compost	1.4	84	3
CO 2.0	compost	2.0	120	4

and compost (CO), with different stocking rates, and control (C) without any fertilization. Slurry and compost were applied on experimental plots with the stocking rates of 0.9, 1.4, and 2.0 livestock units (LU)/ha. Plots were cut depending on stocking rates. Particular treatments are shown in Table 2. Arrangement of plots was done in a completely randomized block design in four replicates. The plot size was 12.5 m². Fertilizers were applied annually as follows: compost in the early spring, half of slurry was applied in the early spring and the second half after the first cut. Slurry was diluted with water in the ratio of 1:3. Before the application, total nitrogen content was determined both in compost and slurry on the basis of which the rate of fertilizers was calculated. Table 3 shows the chemical composition of the organic fertilizers.

Measurements. Sampling was done twice a year, in March (before fertilizers application and cutting) and October (after fertilizers application and cutting) during 2008 and 2013. The samples were taken from a depth of 0.05–0.30 m. Total number of samples was 56 per year (28 in spring and 28 in autumn). Total organic carbon (C_{ox}) was determined by oxidimetric titration (NELSON & SOMMERS 1982), hot-water extractable carbon (C_{hws}) according to KÖRSCHENS *et al.* (1990), and cold water extractable carbon (C_{cws}) according to VÁCHALOVÁ *et al.* (2013). Fractional composition of humic substances (HS), humic acid (HA) and fluvic acid (FA) (ΣHS, ΣHA, and ΣFA) was determined by the short fractionation method (PODLEŠÁKOVÁ *et al.* 1992). Selected trace

elements (Cd, Co, Zn, and Cu) were determined. Contents of total (T) elements were determined after microwave extraction in *aqua regia* (Ethos 1 microwave system, Milestone, Sorisole, Italy). Contents of labile elements were determined in 0.01M CaCl₂ solution (labile form – plant available (PA)) using an AAS spectrometer ContrAA 700 (Analytic Jena, Jena, Germany). Macroelements (Ca, Mg, K, P) were detected using Mehlich III method. Total nitrogen was determined according to Kjeldahl method. For HA isolation, the standard IHSS extraction method was applied (HAYES 1985). Humic acids were purified, dialyzed, and lyophilized at –50°C. The elemental analysis was performed using a CHN analyzer PE 2400 CHNS/O (PerkinElmer, Waltham, USA). Energy-dispersive X-ray spectra were detected by an energy-dispersive X-ray spectrofluorimeter XEPOS (Shimadzu, Kyoto, Japan).

Data analyses. The SPSS 13.0 statistical package for MS Windows (StatSoft 1997) was used for the statistical data analysis and the analysis of variance (ANOVA). Mean statistical differences (95% significance level) were calculated by Tukey's HSD test ($P < 0.05$). Homogeneity of variances was tested by Cochran's C test. For redundancy analysis (RDA), Canoco version 4.5 was applied (TERBRAAK & ŠMILAUER 2002) to elucidate the relationship between the soil chemical properties and the management treatments. Correlation analysis was used to determine the relationship between the organic carbon forms and the elements.

Table 3. Average concentrations of elements in organic fertilizers

Fertilizer	P	K	Ca	Mg	Cd	Zn	Co	Cu	C* (%)
	(mg/kg)								
Slurry	6 447	27 595	25 948	10 637	0.11	295	2.35	36.3	35
Compost	2 535	4 120	21 387	3 687	0.24	195	1.19	20.4	52

*C – total content of carbon determined by loss on ignition method

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RESULTS AND DISCUSSION

The effect of fertilizers on elements. The mean concentrations of trace elements and macroelements in soil are given in Table 4. The macronutrients (except K) were evidently directly influenced by the fertilizer type and the stocking rate. The highest concentration of P, Ca, Mg, and N was detected with CO 2.0 treatment. The measured concentrations of macronutrients were evaluated as good, except the high concentration of Mg, as reported for permanent grassland (FIALA & KRHOVJÁKOVÁ 2009).

The ordination diagram (Figure 1) demonstrates the effect of treatments on the studied soil parameters at $P = 0.05$ significance level and explains 31% of data variability. According to the ordination axis, the treatments can be generally divided into two groups, influenced either by compost (on the right side of the ordination diagram) or by slurry (on its left side). Furthermore, the positive effect of compost on C_{ox} , C_{hws} , HA, FA, HS, N, and macronutrients was documented. Fertilizer type showed a direct effect on total (T) elements contents. Statistically significant effects were recorded for ZnT and CdT contents. Higher values of CoT and CuT were observed by the slurry treatments. Stocking rate had no effect on the concentrations of total elements. Plant available (PA) trace elements were more affected by the type

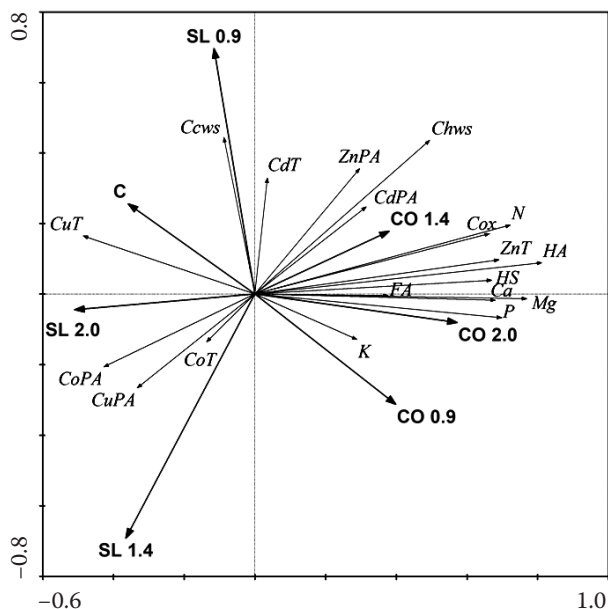


Figure 1. Ordination diagram showing the result of RDA analysis of chosen soil parameters (T – total content of trace element; PA – plant available, labile content of trace element; C – control; CO – compost; SL – slurry)

Table 4. Average concentrations of elements and pH in the slurry (SL) and compost (CO) treatments

Treatment	pH	CaCl ₂	P	(mg/kg)			N (%)	(mg/kg)							
				Ca	Mg	K		ZnT	ZnPA	CoT	CoPA	CuT	CuPA	CdT	CdPA
C	4.8 ^{ab}		53 ^a	1855 ^a	173 ^a	159	0.181 ^{ac}	50 ^b	0.077 ^a	12.8	0.068	23	0.055	0.190 ^a	0.040
SL 0.9	4.8 ^{ab}		59 ^{ab}	2078 ^{ab}	209 ^{ab}	161	0.186 ^{abc}	52 ^b	0.087 ^{ab}	14.2	0.055	23	0.042	0.177 ^a	0.042
SL 1.4	4.7 ^a		55 ^a	1954 ^{ab}	185 ^a	155	0.169 ^c	51 ^b	0.063 ^a	14.6	0.074	22.5	0.065	0.156 ^{ab}	0.032
SL 2.0	4.9 ^{ab}		48 ^a	1944 ^{ab}	195 ^a	162	0.174 ^{ac}	51 ^b	0.092 ^{ab}	14.4	0.092	24.5	0.059	0.113 ^b	0.043
CO 0.9	5.1 ^{ab}		74 ^{ab}	2399 ^{ab}	228 ^{bc}	196	0.195 ^{ab}	61 ^a	0.086 ^{ab}	14.2	0.073	21	0.039	0.136 ^{ab}	0.042
CO 1.4	5.1 ^{ab}		70 ^{ab}	2383 ^{ab}	216 ^b	151	0.191 ^{abc}	63 ^a	0.113 ^b	14.5	0.065	22	0.050	0.154 ^{ab}	0.043
CO 2.0	5.2 ^b		92 ^b	2493 ^b	260 ^c	180	0.203 ^b	63 ^a	0.082 ^{ab}	12.8	0.039	20	0.051	0.164 ^{ab}	0.040
	0.103		0.007	0.027	0.000	0.310	0.002	0.000	0.005	0.101	0.050	0.201	0.175	0.008	0.243

^{a,b,c} values with the same letters are not statistically different within the column $P < 0.05$; C – control, T – total content of trace element, PA – plant available content of trace element

Table 5. Average values of studied carbon forms in the slurry (SL) and compost (CO) treatments

Treatment	C _{ox} (%)	C _{cws} (mg/kg)	C _{hws} (mg/kg)	HS	HA (%)	FA	HA/FA	C/N
C	1.46 ^a	255	617 ^{ab}	0.72 ^{ac}	0.263 ^a	0.458 ^{ac}	0.57 ^a	8.1
SL 0.9	1.59 ^{ab}	259	686 ^a	0.66 ^{ad}	0.300 ^{ab}	0.360 ^b	0.83 ^b	8.5
SL 1.4	1.44 ^a	216	531 ^b	0.62 ^d	0.263 ^a	0.353 ^b	0.75 ^{ab}	8.5
SL 2.0	1.41 ^a	233	576 ^{ab}	0.72 ^{ac}	0.275 ^a	0.445 ^c	0.62 ^{ac}	8.2
CO 0.9	1.58 ^{ab}	241	641 ^{ab}	0.78 ^{cb}	0.335 ^b	0.448 ^c	0.74 ^{ab}	8.1
CO 1.4	1.61 ^{ab}	254	683 ^{ab}	0.76 ^{cb}	0.335 ^b	0.425 ^{abc}	0.80 ^{bc}	8.2
CO 2.0	1.74 ^b	246	692 ^a	0.84 ^b	0.338 ^b	0.500 ^{ac}	0.68 ^{ab}	8.1
P	0.001	0.075	0.041	0.000	0.000	0.000	0.003	0.522

^{a,b,c,d} values with the same letter are not statistically different within the column $P < 0.05$; C – control; HS – humic substances; FA – fluvic acid; HA – humic acid; C_{ox} – total organic carbon; C_{cws} – cold water extractable carbon; C_{hws} – hot water extractable carbon

of fertilizer than by the stocking rate. There were found statistically significant differences in ZnPA and CoPA concentrations between the treatments, while no statistically significant differences were detected in CuPA and CdPA concentrations. It was also evident that CuPA values had similar trend as CoPA values. Generally higher concentrations of elements in the slurry treatments compared to the compost ones were monitored. The mobility of elements was also affected by the soil pH. According to TLUSTOŠ *et al.* (2007), the highest mobility of Cd is at pH 4.4–5.5. In this study, pH values ranged from 4.7 to 5.2, which was not significant for the differences in the trace elements mobility.

The effect of fertilizers on soil organic matter. The studied carbon forms showed differences between the treatments (Table 5). Differences in C_{ox}, C_{hws}, HS, HA, FA were significant. Higher values of all the studied parameters were found by the compost treatments. However, no statistical differences in C_{cws}

between treatments were monitored. KÖRSCHENS (1998) showed that hot water extractable carbon was more sensitive to different management than total carbon. In our experiment, both C_{ox} and C_{hws} were influenced significantly by compost – the organic fertilizer. According to the RDA (Figure 1), the values of C_{cws} were similar to SL 0.9. Average values of C_{hws} were typical for this soil type. Also KOLÁŘ *et al.* (2003) reported similar data and showed that optimum content ranged from 300 to 600 mg/kg. A statistically significant correlation between C_{ox} and C_{hws}, HA, pH, and macronutrients was found. The same trend was monitored for C_{hws} (see Figure 1). Values of C_{cws} did not correspond to other carbon characteristics. KOLÁŘ *et al.* (2009) and DVOŘÁK (2013) studied the labile fractions of soil organic matter and found out that reproducibility of C_{cws} is often chaotic and without any relationship to other components.

Relationship between the organic matter and the elements. Correlation between different labile

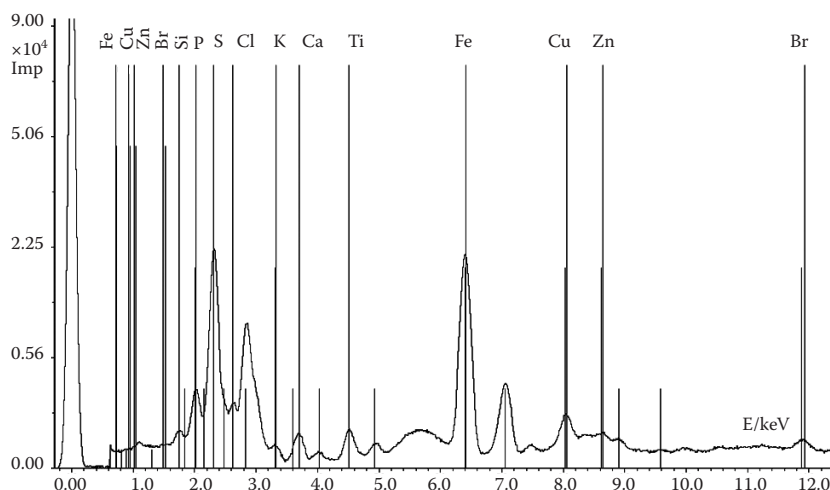


Figure 2. Energy-dispersive X-ray spectra of humic acids isolated from Haplic Cambisol

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Table 6. Correlation between organic carbon forms and concentrations of elements ($n = 28$, $\alpha = 0.05$)

	pH	P	Ca	Mg	K	N	ZnT	ZnPA	CoT	CoPA	CuT	CuPA	CdT	CdPA
C_{ox}	0.5088	0.7088	0.3808	0.6110	0.2855	0.7856	0.4863	0.3865	-0.2929	-0.1981	-0.3997	-0.3153	0.2863	0.4527
C_{cws}	0.1914	-0.1615	0.1401	0.0036	-0.0673	0.2820	0.1223	0.5449	-0.1027	0.2679	0.1708	-0.0990	0.1790	-0.1570
C_{hws}	0.3902	0.5646	0.2024	0.3005	0.3619	0.7371	0.4211	0.3892	-0.2575	-0.2123	-0.4903	-0.4727	0.0669	0.3877
HS	0.6395	0.5567	0.4366	0.5788	0.2753	0.7499	0.7396	0.3645	-0.3951	-0.1121	-0.3363	-0.2548	0.0450	0.3155
FA	0.3989	0.4939	0.4559	0.3987	0.1445	0.4678	0.6467	0.1863	-0.4094	-0.1384	-0.1565	-0.0910	0.0535	0.1438
HA	0.7152	0.7053	0.5136	0.6664	0.3502	0.8386	0.5394	0.4714	-0.1889	-0.0190	-0.4587	-0.3904	0.0108	0.4350

C_{ox} – total organic carbon; C_{cws} – cold water extractable carbon; C_{hws} – hot-water extractable carbon; HS – humic substances; FA – fluvic acid; HA – humic acid; T – total content of trace element; PA – plant available content of trace element; significant results are in bold

carbon forms (C_{ox} , C_{hws} , HS, HA) and elements was ascertained (Table 6). Furthermore, a very strong correlation between pH and organic carbon was detected. As mentioned before, no statistical relationship between C_{cws} and elements was found. It can be concluded that C_{ox} and C_{hws} had a significant effect on the total contents of Zn and Cu and on the plant available forms of Zn, Co, Cu. POSPÍŠILOVÁ *et al.* (2011) described a significant effect of the labile organic matter on trace elements, especially the content of plant available Zn and Cd. The elemental analysis of humic acids showed a lower carbon content and a higher oxygen content in the HA molecule, which indicated young humic acids, with a lower condensation degree. Energy-dispersive X-ray spectroscopy detected the following elements in the HA molecule *in situ*: Fe, Cu, Zn, Ti, Ca, K, S, P, Si, and Br. A high affinity of the humic acids to Fe^{3+} , S^{2+} , and Cu^{2+} was found (Figure 2).

CONCLUSION

Organic fertilizers with graded stocking rates influenced the concentrations of the determined carbon fractions, macronutrients, and trace elements. Treatments with compost showed a greater amount of organic carbon fractions, except the cold water soluble carbon. In the case of macronutrients, the same trend (greater concentrations by the compost treatments) was monitored. We may conclude that the content and quality of the humic substances strongly influenced the total and labile trace elements content in the soil. Furthermore, hot water soluble carbon significantly correlated with plant available forms of Zn, Cu, and Cd.

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