Labile fractions of soil organic matter, their quantity and quality

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ABSTRACT

The objective of the present paper is to contribute to the evaluation of quantity and quality of non-humified part of soil organic matter (SOM). In samples of soil organic matter from the humus profile of Šumava forest soils and forest meadows, taxonomically designated as mor and moder forms, the fractions of labile soil carbon C_{cws}, C_{hws}, C_{PM} and fraction of stable carbon represented by carbon of humus acids C_{HA} and C_{FA} were determined. Organic matter of samples was fractionated according to the degrees of hydrolyzability by two different methods in particle-size fractions of 2.00–0.25 mm and < 0.25 mm. The quality of labile fraction C_{hws} was expressed on the basis of reaction kinetics as the rate constant of biochemical oxidation K_{bio} and rate constant of chemical oxidation K_{chem} of the first order reaction from a reduction in the concentration of C-compounds. The highest values of labile forms of carbon were determined in samples with the least favorable conditions for transformation processes of SOM, and these samples also had the highest content of labile forms in hydrolyses by both methods and the most labile fractions at the same time. The degree of SOM humification was strictly indirectly proportional to the lability of SOM and its hydrolyzability. The quality of labile fraction C_{hws} can be expressed by both K_{bio} and K_{chem} while the sensitivity of K_{bio} is higher but the reproducibility of K_{chem} is better. K_{bio} corresponds with the degree of SOM transformation, K_{chem} with the proportion of C_{PM} in total C_{ox}.

Keywords: primary soil organic matter; labile fraction; quality and quantity; degree of hydrolyzability; oxidation; reaction rate

Soil organic matter is an unusually complicated heterogeneous mixture of organic material mostly composed of plant and microbial residues and it contains mono- to polymeric molecules of organic substances, lignin, various proteins, various polysaccharides (cellulose, hemicelluloses, chitin, peptidoglycans), lipids and another aliphatic material (waxes, fatty acids, cutin, suberin, terpenoids); an attempt at its classification according to chemical composition was made by Kögel-Knabcher (2002). A number of semi-products originate from this basic mixture of primary soil organic matter in the exothermic decomposition process of mineralization as well as in the endothermic synthetic process of humification including the products of humification – fulvic acids, humic acids, humins and their other reaction products, salts of humus acids and organomineral compounds – complex heteropolar salts and adsorption complexes.

Efforts to acquire rapid, relatively cheap information on the properties of SOM in the original condition not influenced by isolation techniques have recently relied on modern instrumental analyses, e.g. on mid-infrared diffuse reflectance spectroscopy (DRIFTS) and near-infrared reflectance spectroscopy (NIRS) and ^{13}C NMR spectroscopy (Capriel 1997) applied for practical determination of soil organic matter quality. The results are little convincing for the time being, similarly like classical criteria, the ratio of humic to fulvic acids or the ratio of extinctions of decalcinated humus substances in an alkaline solution at wavelengths of 400 and 600 μm, so called color quotient $Q_{4/6}$. It is so because besides the diversity of non-humified primary soil organic matter in the category of humic acids and fulvic acids there is a high number of individuals highly differing in properties according to their structure and according to

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their relative molecular weight. Therefore e.g. in sorption processes lower humic acids resemble fulvic acids by their properties rather than higher humic acids.

DRIFTS, NIRS and ^{13}C NMR spectroscopy are however irreplaceable in the scientific study of the chemical structure of SOM fractions (Baldock et al. 1992).

Obviously, an attempt at analytical description of such a complicated mixture is fully unreasonable from chemical aspects even though attractive. The undivided mixture is in natural condition, and therefore its potential description promises a true picture of reality. Unfortunately, the only common trait in the mixture is carbon content but the determination of C_{ox} cannot describe SOM properties important for practice. It is so because the set of organic matter of SOM, which has not undergone humification yet, i.e. primary (un)decomposed matter, has quite different properties than the set of SOM humification products.

The objective of this study is to contribute to the evaluation of the quality and quantity of primary (non-humified) part of SOM.

If we abstract from all secondary functions of SOM in soil, there will remain two basic functions: capacity of mineralization with release of energy for soil micro-edaphon, CO_2 and mineral nutrients. This is a property of the primary part of SOM that may be more or less decomposable. It mostly has sorption properties and only minute or no ion exchange capacity. However, it may also be almost undecomposable, inert, of course in the given soil conditions.

Ion exchange capacity is the second basic function of SOM. It is typical for products of humification that are the more resistant to mineralization, the higher their relative molecular weight and the greater their capacity to form organomineral complexes, in other words: the higher their quality for practice.

In this way, the diverse mixture of SOM can be divided into two large groups at least according to different behavior in mineralization and ion exchange.

The first basic function of SOM, to undergo mineralization, obviously shows that those fractions of SOM are the most valuable that are the least stable, hence easily decomposable. These fractions are currently considered as an important indicator of soil quality (Haynes 2005, Maia et al. 2007). The labile fraction is described in a different way. It may be represented by carbon compounds soluble in hot and cold water, compounds extractable with solutions of various salts, content of soluble proteins, hemicelluloses and sugars and mineralizable organic matter. The lability of organic matter is derived from basal respiration carbon, from the content of amino sugar carbon, microbial biomass carbon, content of particulate organic matter carbon, from fractions of gradual oxidation with $K_2Cr_2O_7$ in 6M, 9M and 12M H_2SO_4, from the content of carbon oxidizable with 15.6 + 33 + 333mM $KMnO_4$ (Blair et al. 1995, Chan et al. 2001, Rovira, Vallejo 2002, 2007).

The degree of stability of plant or other organic material as a potential decomposable substrate also for organic manuring is evaluated in a similar way. It is recommended to use a division into 3 fractions according to stability in acid hydrolysis with 1M and 2.5M H_2SO_4 at 105°C and 0.5–12 h of reaction time (Rovira and Vallejo 2000, 2002, Shirato and Yokozawa 2006). For stability estimation other authors use oxidizable carbon of the material in neutral 33mM $KMnO_4$ (Tirol-Padre and Ladha 2004) or division into 4 fractions according to oxidizability of C compounds with $K_2Cr_2O_7$ in 6M, 9M and 12M H_2SO_4 (Chan et al. 2001).

In general, chemical fractionation of SOM abandons the quantification of the particular types of organic compounds (proteins, amino sugars, amino acids, lipids, etc.) (Appuhn et al. 2004) and is focused on the hydrolysis of polysaccharide structures (Rovira and Vallejo 2000) and on the SOM division into fractions according to different stability in acid hydrolysis and permanganate oxidation (Blair et al. 1995, Paul et al. 2001).

The problem of SOM stability was solved by many authors who studied physical fractionation of SOM (Rethemeyer et al. 2005), biological stability of plant residues and the rate of change of O/N-alkyl C to alkyl C and its hydrophobic character as a cause of biological stability of SOM components (Kögel-Knabcher et al. 1992), the effect of biological capability and capacity on the decomposability of organic material (Baldock 2007) and protective effect of a number of factors on the mineralization decomposition of SOM.

MATERIAL AND METHODS

Samples of soil organic matter were taken from the humus profile of Šumava forest soils, i.e. from horizons of forest floor and humus A horizon underlying them. Description of these samples is shown in Table 1.

Having passed through a 2-mm sieve, air-dried samples were divided into 2 fractions on a 0.25-mm

Table 1. Description of samples of soil organic matter (Němeček 2001)

Sample	Form	Designation of forest floor
1	anhydrogenous	mor-like moder, L, F_a, H_h, A
2		matted mor, L, F_m, H_h (A_h)
3		sod mor, F, H_h, A_h
4	hydrogenous	hydromoder, F_a, H_h (A)
5		fibrous mor, L, O_f, T_f
6		mesic mor, L, O_m

L – litter horizon; H_h – humus horizon of mull; A – humic horizon; F_a – amphigenous matted horizon; A_h – humic forest horizon; F_m – mycogenic matted horizon; O_f – fibrous hydrogenous horizon; T_f – peaty fibrous horizon; O_h – humus hydrogenous horizon; O_m – mesic hydrogenous horizon

sieve: > 0.25 mm with the major part of undecomposed organic matter and fraction < 0.25 mm. In the fraction > 0.25 mm we determined in all samples the categories of hydrolyzability on the basis of the results of hydrolysis in 6M, 9M and 12M H_2SO_4 (Walkley 1947) as modified according to Chan et al. (2001) and categories of hydrolyzability according to Rovira and Vallejo (2000, 2002) as modified by Shirata and Yokozawa (2006). In the fraction < 0.25 mm, besides both tests of hydrolyzability, the fractionation of labile forms of soil carbon was done in all samples by determining hot water soluble C-compounds (C_{hws}) and cold water soluble C-compounds (C_{cws}) according to ordinary methodology and of labile C compounds oxidizable with a neutral 33mM solution of $KMnO_4$ (C_{PM}) according to the method of Blair et al. (1995) as modified by Tirol-Padre and Ladha (2004).

In addition, carbon of humic acids $C_{ox\ HA}$ and carbon of fulvic acids $C_{ox\ FA}$ were determined in the fraction < 0.25 mm after alkaline extraction of samples with a mixture of 0.1 $Na_4P_2O_7 \cdot 10\ H_2O$ and NaOH, and after HA precipitation and FA separation according to the classical method of Kononová and Belčiková (Hraško 1962). The proportion of $C_{ox\ HA}$ + $C_{ox\ FA}$ in total $C_{ox\ tot}$ was calculated. $C_{ox\ HA}$, $C_{ox\ FA}$ and $C_{ox\ tot}$ were determined by wet combustion with $K_2Cr_2O_7$ in accordance with ISO 14235 (1995). At the same time, C_{org} was determined by a combustion technique at 1 095°C in accordance with ISO 10694 (1996).

The quantity of labile organic matter of soil was assessed in the given set of samples by two hydrolytic procedures and three methods (C_{cws}, C_{hws}, C_{PM}). The quality of labile organic matter was investigated only in fractions C_{hws} and C_{PM} because the reproducibility of C_{cws} was very low. The rate constant of their biochemical oxidation, as the first order reaction, is considered as the measure of quality of labile fraction C_{hws} in soil organic matter, which is measured according to our original methodology in an Oxi Top Control Merck vacuum system (Kolář et al. 2006). The lower reproducibility of results of this method, given by different hydrolytic quality of the used inoculum, is solved in this study by replacing biochemical oxidation with chemical oxidation only, and the residual concentration of carbon is used for the calculation of the rate constant when the value of C_{PM} is determined in several time intervals of 1, 3, 6 and 24 h. A comparison of both methods is presented in this study. Lord's test for few-element sets is used to determine the consistency of results of both methods (Eckschlager, Horsák, Kodejš 1980).

RESULTS AND DISCUSSION

A description of the samples of soil organic matter in Table 1 documents that all samples of overlaying SOM belong to the mor form according to the Taxonomic Classification System of Soils of the Czech Republic (Němeček 2001) from forest soils and forest meadows, which is formed in adverse conditions for the decomposition and transformation of soil organic matter, mostly on acid poor-in-minerals soils in the wet and cold climate. Samples 1 and 4 belong to the moder form, which is a transitional form between mor and mull and which is formed in more favorable conditions for the decomposition and transformation of organic residues. Mor-like moder (sample 1) with typical amphigenous matted horizon F_a is approaching mor. Hydromoder (sample 4) develops at increased soil moisture content on soils waterlogged for a long time but not permanently. Sample 2 is from a drier site: mycogenic matted horizon F_m prevails as a sign of markedly slowed down humification. Sample 3 is from a mountain location under the layer of the grass sod with dominance of matgrass. Horizon H_h is viscous. Sample 5 with typical hydrogenous fibrous horizon O_f on Organosol with peaty fibrous horizon T_f with more than 2/3 of undecomposed organic matter represents the biologically extraordinarily little active SOM. Sample 6 is poorly aerated mor in conditions of permanent waterlogging with the major part of hydrogenous mesic horizon O_m from dead parts

Table 2. Fractionation of organic matter carbon in samples 1– of particle size < 0.25 mm

Sample	C_{org} (%)	C_{ox} (%)	$C_{org} - C_{ox}$ (%)	C_{hws} (mg/g)	C_{cws} (mg/g)	C_{PM} (mg/g) 1 h	$C_{ox\ HA}$ (mg/g)	$C_{ox\ FA}$ (mg/g)	HA : FA
1	8.6	7.7	0.9	2.9	0.4	11.7	1.7	5.7	0.30
2	9.3	8.1	1.2	3.1	0.4	13.9	0.5	2.8	0.18
3	36.5	32.0	4.5	11.3	1.9	63.7	5.3	19.6	0.27
4	6.5	5.1	1.4	0.9	0.4	7.9	2.1	6.2	0.34
5	9.4	7.9	1.5	4.7	0.4	19.3	0.2	1.8	0.11
6	2.7	2.4	0.3	0.5	0.1	5.4	0.3	1.3	0.23

of mosses and sedges. Surprisingly, conditions for the decomposition of organic matter are a little more favourable there because the groundwater level is not stable as it fluctuates.

Table 2 shows that the interval of the values of C_{ox} and/or C_{org} is very wide (2.7–36.5% C_{org}) and this is the reason why the analytical data on the fractions of labile carbon C_{hws}, C_{cws}, C_{PM} and on stable forms of carbon $C_{ox\ HA}$, $C_{ox\ FA}$ do not seemingly correspond to the taxonomy of samples from Table 1. In Table 5 these values are expressed as the percent of total C_{ox} and the correlations are already quite evident. The sample that taxonomically corresponded to the highest activity of transformation processes of SOM, i.e. sample No. 4, has the relatively lowest values of labile carbon fractions while sample No. 5 with the taxonomically assumed lowest activity has the highest values of labile carbon. The content of the fraction of humus acids, which represents the stable form of carbon, is the highest in sample 4 and the lowest in sample 5. From this aspect labile fractions of SOM can be considered as unused material in transformation processes, not as a sign of the high level of hydrolytic processes in the given soil environment. The worse the conditions for transformation activity in the given set of samples, the higher the content of labile fractions of SOM was, while the relationship was inverse in the stable fraction of humus acids. This relationship was described better by determination of C_{hws} than by C_{PM}; determination of C_{cws} brought about only chaotic results without any relationship. The difference between C_{org} and C_{ox} in the set of samples ranged in the interval of 0.32–4.47% (Table 1), which corresponds to the interval of 11.2–26.9% if expressed as % C_{ox} (Table 5).

The hydrolyzability of larger particle-size samples could be expected to be lower from the purely chemical aspect than in the fraction < 0.25 mm

Table 3. Distribution of organic matter in samples 1–6 of particle size 2.00–0.25 mm and < 25 mm (in % of total C_{org} in dry matter) into classes according to hydrolyzability according to Chan et al. (2001)

Sample	Fraction							
	1	2	3	4	1	2	3	4
	particles (mm)							
	2.00–0.25				< 0.25			
1	25	30	12	33	23	28	20	29
2	30	42	10	18	30	40	12	18
3	27	37	19	17	25	26	18	31
4	25	35	15	25	22	30	18	30
5	44	38	6	12	40	35	12	13
6	41	30	10	19	35	29	14	22
$LSD_{0.05}$	6.2	5.0	1.9	3.8	6.0	5.7	2.8	4.2

Fraction: 1 – 6M H_2SO_4; 2 – 9M–6M H_2SO_4; 3 – 12M–9M H_2SO_4; 4 – C_{org} – 12M H_2SO_4

Table 4. Distribution of organic matter in samples 1–6 of particle size 2.00–0.25 mm and < 0.25 mm (in % of total C_{org} in dry matter) into classes according to hydrolyzability according to Shirato and Yokozawa et al. (2006)

Sample	Fraction					
	labile	partly resistant	resistant	labile	partly resistant	resistant
	particles (mm)					
	2.00–0.25			< 0.25		
1	16	39	45	13	40	47
2	20	27	53	18	26	56
3	12	40	48	11	41	48
4	11	23	66	4	17	79
5	36	28	36	30	24	46
6	21	39	40	15	40	45
$LSD_{0.05}$	2.5	8.2	6.7	1.8	7.4	5.9

because the processes of diffusion and equilibrium establishment must be more rapid in disintegrated material. But the reality is just opposite: the 2.00–0.25 mm fraction has a higher proportion of more labile fractions 1 and 2 than the particle-size fraction < 0.25 mm in all SOM samples of the set. Obviously, the larger particle-size fraction is mostly composed of yet unhydrolyzed organic material in these adverse soil conditions while in the particle-size fraction < 0.25 mm transformation processes of SOM that are connected with an increase in stability in relation to hydrolysis have taken place at least partly (Table 3). Generally, the determination of hydrolyzability by another method provided practically the same results but the absolute values of fractions are naturally different (Table 4). Table 3 shows that in the particle-size fraction < 0.25 mm the differences between labile fraction 1 and stable fraction 4 of hydrolyzability are in the interval of –27 to +8% of total C_{org} and the samples can be arranged according to hydrolyzability into this series:

5 > 2 > 6 > 1 = 3 > 4

This series is practically identical with the series of samples arranged according to an increase in the content of humus acids (Table 5):

5 < 2 < 6 < 3 < 1 < 4

and very similar to the series arranged according to the content of C_{hws} in % C_{ox}:

5 > 2 > 1 > 3 > 6 > 4

and also ranked according to C_{PM} in % C_{ox}:

5 > 6 > 3 > 2 > 4 > 1

In most cases, samples 5, 2 and 6 represent samples with limited transformation of SOM while samples 4 and 1 are those with more profound transformation and stabilization of the labile fraction of SOM. This conclusion complies with sample taxonomy.

Applying the second method, the results of hydrolyzability of organic matter of samples 1–6 do

Table 5. The values of the difference ($C_{org} - C_{ox}$); C_{hws}; C_{cws}; C_{PM} and the sum of $C_{ox\ HA} + C_{ox\ FA}$ expressed as the percentage of C_{ox} in samples 1–6

Sample	$\frac{C_{org} - C_{ox}}{C_{ox}} \times 100$	$\frac{C_{hws}}{C_{ox}} \times 100$	$\frac{C_{cws}}{C_{ox}} \times 100$	$\frac{C_{PM}}{C_{ox}} \times 100$	$\frac{C_{or\ HA} + C_{ox\ FA}}{C_{ox}} \times 100$
1	11.2	3.8 ± 0.8	0.6 ± 0.3	15.1 ± 1.7	9.7 ± 2.6
2	15.7	3.9 ± 0.8	0.5 ± 0.4	17.2 ± 1.9	4.1 ± 1.8
3	12.4	3.1 ± 0.6	0.5 ± 0.3	17.7 ± 2.1	7.8 ± 2.4
4	26.9	1.9 ± 0.5	0.8 ± 0.6	15.5 ± 1.8	16.3 ± 5.0
5	18.5	5.9 ± 1.4	0.6 ± 0.4	24.4 ± 2.7	2.5 ± 0.9
6	13.2	2.3 ± 0.5	0.4 ± 0.3	22.1 ± 2.3	6.6 ± 1.6

Table 6. The mean $\hat{a}_1$ of the rate constants of biochemical oxidation K_{bio}; mean $\hat{a}_2$ of the rate constants of chemical oxidation K_{chem}; range of the results of $R_{K\,bio}$ and $R_{K\,chem}$ of labile fraction C_{hws} as a picture of the quality of SOM labile components

Sample	$\hat{a}_1 K_{bio}$ (24 h)	$\hat{a}_2 K_{chem}$ (24 h)	$R_{K\,bio}$	$R_{K\,chem}$	
1	0.025	0.185	0.020	0.005	
2	0.065	0.170	0.030	0.005	number of parallel determinations: 5
3	0.030	0.150	0.020	0.005	critical value of Lord's test for $\alpha = 0.05$: $u_\alpha = 0.306$
4	0.010	0.205	0.015	0.010	$u = \frac{\lvert \hat{a}_1 - \hat{a}_2 \rvert}{R_1 + R_2} = 2.91$
5	0.075	0.050	0.045	0.005	
6	0.060	0.080	0.035	0.005	

not differ from those of the first method, and the series according to the proportion of labile fraction is as follows (Table 4).

5 > 2 > 6 > 1 > 3 > 4

The rate constant of biochemical oxidation K_{bio} in this set of samples ranged in the interval 0.010–0.075 and was the lowest in samples with more profound transformation of SOM. The rate constant K_{chem} in this set of samples was in the interval 0.050–0.205, mostly in a remarkably narrow range R = 0.005 while the range of K_{bio} was in the interval 0.015–0.045. However, the value of K_{chem} did not correspond to the degree of SOM transformation; it very exactly corresponded to the percentage proportion of C_{PM} in total C_{ox}. We have failed to explain it until now. We are convinced that C_{hws} is more sensitive than C_{PM} to characterize labile fractions of SOM because in the given set of samples C_{hws} accounted for only 1.9–3.9% C_{ox} whereas C_{PM} accounted for 15.1–24.4% C_{ox} (Table 5). This is the reason why K_{chem} does not react so sensitively as K_{bio}. It is also documented by quite marked differences in the range R (Table 6). The mathematical and statistical evaluation (Table 6) shows that the results of K_{bio} determination are statistically significantly different (at $\alpha = 0.05$) from the results of K_{chem} determination and the differences in means x = cannot be explained by random errors.

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