REVIEW

Methods for determination of labile soil organic matter: An overview

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Abstract

Soil organic matter (SOM) can be divided into three main pools: labile, stable and inert. Research over recent years has focused on the labile fraction (LF), as it is considered a quickly reactive indicator of soil productivity and health, and important as a supply of energy for soil micro-organisms. A wide spectrum of analytical methods has been used to determine and/or evaluate LF, based on physical, chemical and biochemical principles. The advantages and disadvantages of each technique are explored in this work, but none of the methods can determine LF sufficiently, either because a part of the LF is not involved or because further characterisation is missing. Although analytical methods are widely used to evaluate changes in soil management or organic carbon turnover, the practical question of the quantity and quality of SOM cannot be answered completely. It is also suggested that future research should focus on the interactions among SOM fractions and their better chemical and functional characterisation. It is possible to use a combination of the analytical methods reviewed here in order to accomplish this objective.

Key words: soil organic matter; organic carbon; labile fraction; decomposition; analytical method

List of abbreviations

BOD — Biochemical Oxygen Demand
BSR — Basal Soil Respiration
C_{CWS} — Cold Water-Soluble Carbon
C_{HWS} — Hot Water-Soluble Carbon
COD — Chemical Oxygen Demand
C_{PM} — Carbon oxidised with potassium permanganate
DOC — Dissolved Organic Carbon
DOM — Dissolved Organic Matter
DON — Dissolved Organic Nitrogen
LF — Labile Fraction
LtF — Light Fraction
MBC — Microbial Biomass Carbon
OC — Organic Carbon
POC — Particulate Organic Carbon
SOM — Soil Organic Matter
TOC — Total Organic Carbon
TON — Total Organic Nitrogen
WEOC — Water-Extractable Carbon
WEOM — Water-Extractable Organic Matter
WSC — Water-Soluble Carbon

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INTRODUCTION

Soil organic matter (SOM) is considered an important part of soil for its high contribution to soil productivity. Generally, SOM contains two main fractions: humic substances and labile soil organic matter.

Humic substances have been found to be a stable material, specific to each soil and not markedly changing over decades of soil use (Siewert 1989, Stevenson 1994). There is also a new paradigm of what humic substances really are (Piccolo 2002). They have been the focus of pedological research for more than 50 years (Najmr 1958, Kononovová and Belčiková 1961, Flaig et al. 1975, Stevenson 1982), but, in the last decades, more attention has been paid to the labile SOM pool (Körschens et al. 1990, Blair et al. 1995, Kubát et al. 1999, Gregorich et al. 2003, Kolář et al. 2009), which has been acknowledged as a good indicator of soil quality and environmental health (Ghani et al. 2003, Haynes 2005, Laik et al. 2009). It is more sensitive to tillage, manuring, fertilisation, crop rotation and other interventions than total organic matter (Bongiovanni and Lobartini 2006, Heitkamp et al. 2009). Furthermore, the effects of changes in soil management are observable sooner in the labile SOM pool than in the total SOM (Lee et al. 2009).

The aim of this work is to introduce a new approach to SOM classification and to give an overview of principles and methods for labile pool separation, quantification and evaluation.

SOIL ORGANIC MATTER CLASSIFICATION

Former classifications were based on the extraction procedure of the different chemical fractions (Tjurin 1937, Najmr 1958), and the morphological (Scheffer and Ulrich 1960) and visual properties (colour) (Stevenson 1982) of SOM or of its origin (field, forest, soil type) (Alexandrovová 1970). The function and importance of the above mentioned pools were only secondarily derived indicators.

However, a recent classification, which uses function as an important criterion, differentiates:

a) **labile** SOM – a quickly reactive labile organic matter, which provides energy and nutrients for soil micro-organisms and releases part of the nutrients for plant usage. Its half-life is between days and few years. It provides short-term organic matter turnover during the year;

b) **stable** SOM – a reservoir of less decomposable organic matter. The main and the most important function of this pool is its cation-exchange capacity. This pool is often bounded in organic-mineral aggregates. Its half-life is between years and decades;

c) **inert** SOM – an amost non-reactive organic matter which affects the physical properties of the soil. It has a potentially low sorption capacity. This pool is physico-chemically protected against decomposition. Its half-life is between decades and centuries.

Labile SOM

Definition and origin

Various approaches use different terms and definitions of the labile SOM pool (labile fraction (LF)). Previous classifications, as mentioned above, never used the concept of LF. However, some similarities in the character of former concepts can be observed and the evolution of the definition of the fraction approximating to the LF and the final emergence of the term itself, are shown in Table 1. The recent SOM classification used in the Czech Republic is shown in Fig. 1.

Table 1. Evolution of the labile soil organic matter definition

| Term	Reference	Description
Non-humic substances	Tjurin (1937)	lignin, cellulose, hemicelluloses, low-molecular proteins, products of decomposition – organic acids, amino acids
Nutritive humus	Najmr (1958)	hydrolysable organic matter
Non-specific humic substances	Kononovová (1963)	products of organic residues decomposition and products of microbial resynthesis
Active fraction	Paul (1984)	non-biomass active components, temporary pool for nutrients
Primary organic matter	Schulz and Klimanek (1988)	non-humified organic matter
Labile fraction	Biederbeck et al. (1994)	readily-decomposable organic matter with temporal fluctuations
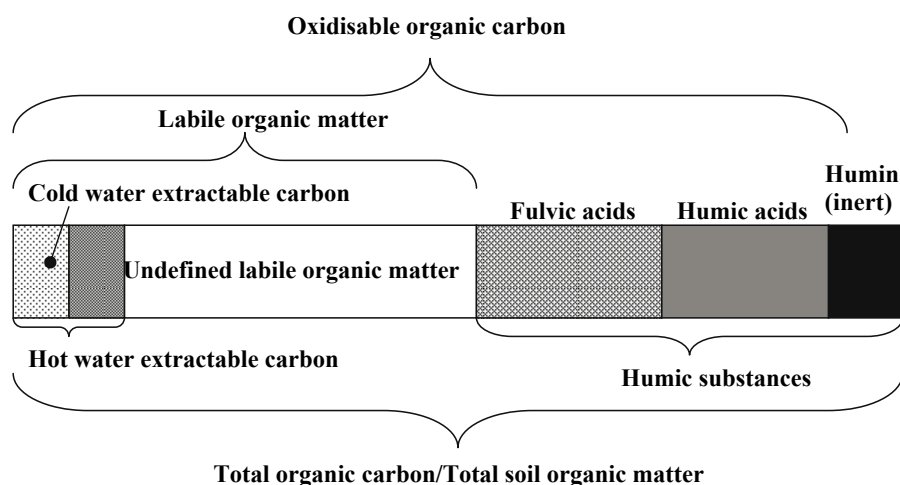
Fig. 1. Fractionation of soil organic matter by classical methods

Composition and characteristics

The LF consists of: micro-organisms, plant and soil fauna residues at different levels of decay and the products of their decomposition, easily decomposable non-humic organic substances such as carbohydrates, polysaccharides, proteins, organic acids, amino acids, waxes, fatty acids, and other non-specific compounds (Poirier et al. 2005).

The rate of decomposition or mineralisation depends on two conditions: firstly, the presence of relevant soil condition – mainly, moisture, temperature, porosity and pH – which support or inhibit the decomposition process, and secondly, the nature of the compounds present and their availability for micro-organisms affected by their chemical structure and composition (Capriel 1997).

Analytical methods focus on these properties from various points of view.

METHODS OF ANALYSIS

Two types of analytical methods are most frequently used: i) Physical, chemical and biochemical analysis of the non-living substrate and ii) determination of the microbial activity. Table 2 presents the methods selected for separation and/or evaluation of the LF. The aim is not to identify the individual chemical, but to understand the purpose and function of these fractions in soil as a whole (Loveland and Webb 2003).

Physical methods

Particulate organic carbon (POC)

By definition, POC consists of pieces of plant or fauna residues, but according to Krull et al. (2006), it may sometimes also contain inert charcoal. In addition, there is no evidence to explain why free organic matter in soil solution sediments in particles of a specific size. These issues illustrate the inconsistency of the POC analysis.

Densitometric separation

Density fractionation is based on the different densities of mineral fraction (usually over 2 g.cm^{-3}) and organic matter (usually below 1.6 g.cm^{-3}). The light fraction (LtF) contains free organic matter, medium or occluded fractions, aggregates (where organic matter and minerals are slightly bound) and heavy fractions (where organic matter is strongly bound to minerals) (Cambardella and Elliot 1993, Alvarez et al. 1998).

The idea that SOM is protected against decomposition when bound into organo-mineral aggregates was proposed by Körschens (1980). It was considered that organo-mineral aggregates contain humic substances. However, further work has shown that low-molecular compounds, especially saccharides, also create highly stabilised sorption complexes (Schulten and Leinweber 1999).

Table 2. Methods for labile soil organic matter analysis. Quality = decomposability or other related characteristics. Quantity = size of the fraction, amount of organic matter in fraction. For the abbreviations used see List of abbreviations.

Method	Term	Principle	Reference	Fractions	Advantages (+) Disadvantages (–)
Physical					
Size fractionation	POC	particles not passed by wet-sieving	Gregorich et al. (2006), Baldock (2007)	TOC in size fraction >53 µm	+ easy performance – insufficient knowledge about properties and functions – possibility to involve inert charcoal – only quantity characterisation
Densitometric separation	LtF	Separation in heavy-liquid solution e.g. sodium polytungstate (NaPT), size of the fractions determined by TOC	Alvarez et al. (1998)	Light <1.6 $g.ml^{-1}$ Medium = 1.6–2.0 $g.ml^{-1}$ Heavy >2.0 $g.ml^{-1}$	+ easy performance – only quantity characterisation
			Rovira and Vallejo (2003)	Light <1.6 $g.ml^{-1}$ Occluded I <1.6 $g.ml^{-1}$ + ultrasonic dispersion Occluded II = 1.6–1.8 $g.ml^{-1}$ Occluded III = 1.8–2.0 $g.ml^{-1}$ Dense >2.0 $g.ml^{-1}$	+ more finely distinguished fractions – only quantity characterisation
DOM	DOC/DON	TOC/TON dissolved in soil solution	Giesler and Lundström (1993)	TOC/TON in field moist soil sample after centrifugation by 16,000 g for 30 min. at 4 °C (large stones removed, aggregates broken by hand)	+ determination the real level of organic matter distributed in water solution + easy performance – only quantity characterisation
WEOM	C_{HWS}	Hot water-extractable carbon	Körschens et al. (1990)	TOC in extract: 60 min. gentle boiling in distilled water	+ easy performance, well reproducible results – does not involve complete LF – only quantity characterisation
			Ghani et al. (2003)	TOC in extract: 16 h at 80 °C shaking in distilled water (sequential after C_{CWS} extraction)	
	C_{CWS}	Cold water-extractable carbon	Ghani et al. (2003)	TOC in extract: 30 min. at 20 °C shaking in distilled water	+ easy performance – worse reproducible results – involve only very narrow spectrum of SOM – only quantity characterisation
	WSC	Water-soluble carbon	Tirol-Padre and Ladha (2004)	TOC in extract: 1 h at 20 °C shaking in distilled water, next centrifugation, filtration	

Method	Term	Principle	Reference	Fractions	Advantages (+) Disadvantages (–)
Chemical					
	C_{PM}	$KMnO_4$	Blair et al. (1995)	Fraction I = C oxidised by 333 mM $KMnO_4$ Fraction II = TOC – fraction I	+ easy performance − only quantity characterisation
Oxidation	Modified Walkley-Black Method	C oxidised by 0.167 M $K_2Cr_2O_7$ with addition of H_2SO_4, retitration with 1 M Fe^{II+}	Chan et al. (2001)	Fraction 1 = 6 M H_2SO_4 Fraction 2 = 9 M H_2SO_4 – F1 Fraction 3 = 12 M H_2SO_4 – F2 Fraction 4 = TOC – F3	+ characterises both quality and quantity + sensitive distinction of fractions − work with dangerous chemicals − worse reproducibility of results
	Sequential oxidation	COD with $K_2Cr_2O_7$ + H_2SO_4 mixture (45 min. at 125 °C), retitration with 0.1 M Fe^{II+}	Strosser (2008)	Fraction 1 = 0.0167 M $K_2Cr_2O_7$ + 2.25 M H_2SO_4 Fraction 2 = 0.0333 M $K_2Cr_2O_7$ + 4.50 M H_2SO_4 Fraction 3 = 0.0500 M $K_2Cr_2O_7$ + 6.75 M H_2SO_4 Fraction 4 = 0.0667 M $K_2Cr_2O_7$ + 9.00 M H_2SO_4	+ characterise both quality and quantity + good reproducibility of results + dangerous chemicals is in low concentration − less sensitive distinction of fractions
Acid hydrolysis	Decomposable and resistant plant material	TOC in H_2SO_4 hydrolyzate	Rovira and Vallejo (2000), Shirato and Yokozawa (2006)	Labile Pool I = 2.5 M H_2SO_4 (30 min. at 105 °C) Labile Pool II = 13 M H_2SO_4 (20 °C overnight, next dilution to 1M H_2SO_4, 3 h at 105 °C) – LP I Recalcitrant Pool = TOC – LPII	+ both quality and quantity characterisation + very sensitively distinguish fractions + correlated to RothC model + suitable for various substrates
		TOC in HCl hydrolyzate	Silveira et al. (2008)	Hydrolysable in 1 M HCl Hydrolysable in 6 M HCl	− less suitable for organic substrates
Biochemical					
Micro-organism activity	MBC	C difference between fumigated and non-fumigated soil sample	Vance et al. (1987)	Fumigation with chloroform for 24 h, next extraction with 0.5 M K_2SO_4 for 2 h (shaking), C determined by COD or TOC	+ smart concept for determination of micro-organism amount − no determination of enzymes activity

Method	Term	Principle	Reference	Fractions	Advantages (+) Disadvantages (−)
Microorganism activity & substrate quality	BSR	CO_2 evolved from soil during incubation	Novák and Apfelthaler (1964)	20-hour incubation at 28 °C, evolved CO_2 estimated interferometrically	+ true mineralisable organic matter + needs short time + both quality and quantity characterisation − only most labile compounds are mineralized
	Mineralizable C	CO_2 evolved from soil during incubation	Majumder et al. (2007)	24-day incubation at 25 °C, evolved CO_2 trapped in 1.0 M NaOH, surplus of alkali titrated with 1.0 M HCl	+ true mineralizable organic matter + both quality and quantity characterisation − needs long time
	Mineralizable N	NH_4^+ evolved during anaerobic incubation	Keeney and Bremner (1966)	7-days incubation at 40 °C, NH_4^+ determined as TON	+ both quality and quantity characterisation − only most labile compounds are mineralized
	BOD, k_1	biochemical oxygen demand and reaction rate constant	Kolář et al. (2003)	5-day BOD_5 determined manometrically, calculation of reaction rate constant	+ easy performance + both quality and quantity characterisation − expensive equipment

This method takes into account neither chemical composition nor structure. The LF should not correspond only to the LtF. It is recommended that additional analyses be carried out in order to obtain a more accurate characterisation of the organic matter contained in LtF. Densitometric fractionation is more suitable for comparison of the disturbing effects in soil profile, caused by tillage or plant roots (Oades 1984, Gregorich and Ellert 1993).

Dissolved organic matter and water-extractable organic matter

The term *dissolved organic matter* (DOM) will be used within the following text for organic matter naturally dissolved in soil-water solution (mainly saccharides, amino acids, aminosugars), and the term *water-extractable organic matter* (WEOM) for organic matter extracted from soil under various laboratory conditions. Thus, WEOM contains a wider spectrum of extracted compounds, such as hemicelluloses (Balaria et al. 2009), but neither DOM nor WEOM involve the whole spectrum of available substrates for microorganisms.

The content of WEOM varies according to the modified extraction procedures such as extraction solvent, shaking time, temperature, soil preparation and final titration (Körschens et al. 1990, Zsolnay and Gorlitz 1994, Ghani et al. 2003, Tirol-Padre and Ladha 2004). These modifications make comparison of the results rather complicated. The resulting effects of the chosen conditions are thoroughly discussed by Jones and Willet (2005). The main difference appears to be between hot- and cold-water extractions, which has led to their individual definitions, viz: the hot water-soluble carbon C_{HWS} (Körschens et al. 1990) and the cold water-soluble carbon C_{CWS} (Ghani et al. 2003).

A high content of DOM or WEOM is not necessarily beneficial in all cases of soil analysis. This effect can be caused by a good supply of labile organic matter but also by poor microbial activity and limited mineralisation (Hilli et al. 2008).

Despite this disadvantage, DOM and WEOM are widely used methods due to their ease of application and good reproducibility.

Chemical methods

Oxidation

Wet oxidation is a very popular method for the determination of organic matter content in soil. Chan et al. (2001) modified the classic Walkley-Black (1934) oxidation method (in Eastern Europe

according to Tjurin 1951), dividing SOM into four fractions with different lability. Strosser (2008) has proposed a similar method called "sequential oxidation". Neither method oxidizes organic carbon (OC) completely, but only about 90% in the sequential oxidation procedure and about 75% in the modified Walkley-Black procedure. This disadvantage is not significant because the unoxidized percentage of carbon is represented by the most stable OC, which can be calculated as: total OC less oxidizable C. The size of the four fractions in "sequential oxidation" is strictly in proportion to the power of the oxidation agent used. This fact makes the method inapplicable. The increasing popularity of the modified Walkley-Black procedure is well documented. (Mills and Fey 2004, Majumder et al. 2007, 2008, Xavier et al. 2009).

The LF can also be measured using neutral potassium permanganate as an agent (Blair et al. 1995). Depending on the concentration used, this fraction includes approximately 8–14% of total organic carbon (TOC). Although early works considered C_{PM} a susceptible indicator with a good response to the changes in SOM, later reports have found serious imperfections. The value of C_{PM} depends on the TOC, and moreover, lignin undergoes permanganate oxidation much more easily than cellulose, while cellulose is more susceptible to microbial decomposition (Tirol-Padre and Ladha 2004). C_{PM} includes a wider spectrum of organic matter than WEOM, but the character of this matter has not been sufficiently investigated (Skjemstad et al. 2006). It is certain that C_{PM} has small relevance to the respired soil OCs, which contain mainly saccharides (Mendham et al. 2002). Despite this, some authors recommend estimation of the biologically active carbon pool using a very dilute $KMnO_4$ solution (concentration 2.5 mM) (Dell 2009).

Additional oxidizers are sometimes applied; for instance, hydrogen peroxide (Leifeld and Kogel-Knabner 2001) or sodium hypochlorite (NaClO) (Zimmermann et al. 2007) which oxidizes more OC without bonds to the minerals.

Acid hydrolysis

Hydrolysis with mineral acids simulates the stability of SOM against hydrolytical decomposition caused by extracellular enzymes of soil microorganisms. According to Rovira and Vallejo (2000) three-step H_2SO_4 hydrolysis is more extensive when combined with the Rothamsted Carbon Model application (Shirato and Yokozawa 2006).

It is considered that H_2SO_4 is more effective than HCl in hydrolysis of organic matter, especially plant tissues (Plante et al. 2006).

Biochemical methods

Microbial Biomass Carbon (MBC)

Soil MBC estimation is used and acknowledged as an advanced method for evaluation of LF (Carter 1986, Sparling 1992). MBC does not depend on the actual degree of activity in microbial communities and can be used to derive the rate of the mineralization process (Vance et al. 1987). Nevertheless, it does not take into account the species composition of these microbial communities or their enzymatic capacity (Adamczyk et al. 2009).

Most papers emphasize the MBC as an important indicator for useful and reliable results. However, one report shows the limitations of the use of MBC (Broos et al. 2007) in extreme cases where spatial oscillation of field conditions calls for a too large number of samples.

Soil respiration and biochemical oxygen demand

The LF can be measured as carbon dioxide released by micro-organisms in a respiration test. The amount of CO_2 is determined by titration or manometrically. The work of Novák (1964, 1965, 1966) should be mentioned here, as the development and modification of the respiration test were carried out in what was then Czechoslovakia. With the respiration test the disadvantages of both MBC and WEOM can be avoided.

Kolář et al. (2003) have proposed another method for evaluation of the quality of LF based on the *biochemical oxygen demand* (BOD) procedure. The amount of CO_2 is measured manometrically and recorded on a time scale (360-times during one sample/replication); thus the reaction kinetics can be observed and the reaction rate constant derived. In this way, both the quantity (the amount of CO_2) and the quality (the reaction rate constant) characterisation of the LF can be established. In addition, the method can be applied to the WEOM fraction, although the quality determination is less pronounced, as WEOM contains compounds with a narrow frame of reaction rate constant (Heitkamp et al. 2009). Moreover, high sensitivity to the quality of inoculation makes for oscillating results and reduces reproducibility. Researchers are also discouraged by the long time needed for incubation.

Currently, there is a vast choice of analytical methods. However, none of them provides a determination of the complete LF without the need for additives. There is still a large gap in the understanding of what the specific function of each fraction of SOM is.

THE USE OF METHODS

The LF determination is mainly used for: i) evaluation of SOM quality; ii) evaluation of the efficiency of sustainable farming (Wang et al. 2009); iii) a comparison of different soil managements or treatments (Blair 2006, Pajares et al. 2009); iv) understanding the decomposition processes in soil and related energy and nutrient flows (Jabro et al. 2008), and v) measurement of carbon sequestration in soil (Berg et al. 2009, Prechtel et al. 2009).

The LF is considered an indicator of the estimation of the SOM content and its changes (Carter 2002). LF changes relatively quickly with alterations in soil management (Chatterjee and Lal 2009, Lopez-Fando and Pardo 2009, Melero et al. 2009). However, this does not apply to the SOM content. Additionally, the SOM content is not a relevant indication (Kasozi et al. 2009). High SOM content in soil with a high TOC value can be blocked in the inert form or the mineralization process can be limited (typical for highlands, acid or permanently waterlogged soils) (Barriuso et al. 1987, Kolář et al. 2006). On the other hand, the reduction of TOC under the critical limit affects soil properties and productivity very negatively and the balanced organic matter turnover is necessary for sustainable soil management and carbon sequestration is advantageous for the environment (Mullen et al. 1999, King et al. 2005, Stewart et al. 2008). Unclear interpretation of a result when using only TOC determination can be avoided with the use of LF analysis.

The analytical methods for labile SOM determination presented above are widely accepted. Although the fundamental principle for SOM defines it as heterogeneous, SOM shows homogenous behaviour under some of the methods (viz. modified Walkley-Black oxidation, respiration tests and BOD). In the author's opinion, this finding can be explained by the fact that, if a high number of different compounds are mixed (as in SOM), the resulting matter can behave similarly to homogenous material.

CONCLUSION

Despite the large number of methods for labile SOM evaluation, the farmers' need to know the amount and quality of organic matter in their fields still cannot be satisfied completely. Two approaches appear to provide an answer: 1) the methods reviewed can be used with the lack of certainty that the SOM fraction plays the key role in soil productivity or 2) the results of long-term field experiments under defined conditions can be used without an investigation of the real processes in the soil. Using each option separately is evidently inadequate.

The objectives of future research should be the investigation of relations between the individual fractions, and their mutual transformations. An improved characterisation – both chemical and functional – of the individual fraction is needed (Gregorich et al. 2006, Broos et al. 2007, Helfrich et al. 2009, Prechtel et al. 2009).

A new approach should be proposed for measurement of the intensity of carbon mineralization and the sequestration process. It is suggested that this new approach should be based on a combination of the analytical procedures presented, applied on a tested area at repeated time periods.

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