

Chapter 2

Micromorphology Techniques for Soil Organic Carbon Studies

Rosa M. Poch and Iñigo Virto

Abstract Most research about soil organic C (SOC) has dealt with its composition, fractionation, degree of stability and turnover time, through analyses that involve the complete or partial destruction of the soil structure. Different fractionation techniques on soil aggregates have produced information about the relationships between SOC, minerals and soil structure. There is still a gap between aggregates or organo-mineral associations isolated from the soil matrix and the soil structure and organic matter distribution in the field. Micromorphology and micromorphometry can be very useful to fill this gap. Classical micromorphology is capable of producing true-scale images of the 2D porosity of undisturbed soils. The study of soil organic matter (SOM) through light microscopy is however limited by the isotropic nature of most soil organic components. A set of new techniques developed more or less recently exist that can complement the information obtained using classical micromorphology. Fluorescence microscopy, SEM and microanalyses applied to undisturbed samples provide a more detailed information on the arrangement of soil components, although the results do not fully provide information about the distribution of SOM types. TOF-SIMS, NanoSIMS, FTIR microscopy and XANES through synchrotron facilities are able to yield information about the location of SOM functional groups and microorganisms in relation to soil structure, and thus are promising for associating specific microsites and SOM characteristics with different degrees of activity or stability. In this work the fundamentals and usefulness of these techniques for SOM studies are described.

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Introduction

An intrinsic soil characteristic is its spatial variability. This variation is not continuous across the scales, but occurs at abrupt transitions (Burrough 2006), forming the soil cover pattern where the main scale steps are the individual compounds, the materials (intrapedal associations and pedality), the horizon volumes, and the soilscales (Targulian and Goryarchkin 2004; Ruellan 2000; ISSS, ISRIC, FAO 1998). These hierarchy levels, structurally organised, have been proposed by Lin (2003) to soil hydrology, and by Hoosbeek and Bryant (1992) to the study of pedogenetic processes.

In the last two decades progress has been made in the knowledge of SOM composition, stability and reactivity (e.g. Lopez-Capel et al. 2005; Hernández-Soriano et al. 2013; Semenov et al. 2013) and in soil carbon mapping (e.g. Chen et al. 2000; Mueller and Pierce 2003; Simbahan and Dobermann 2006). The soil carbon spatial variability at the scale of horizons and landscapes is taken into account when mapping soil carbon stocks or determining carbon distribution in horizons (e.g. Liu et al. 2012), but most of the methods looking at SOC at the level of material or individual compounds are disruptional, i.e. they disturb the original soil structure by fractionation procedures (von Lützow et al. 2007; Sohi et al. 2010).

While the fractionation of the soil organic fraction (SOM) by isolation of different SOM moieties based on chemical characteristics can render useful information on the SOM characteristics, the functional representativeness of these fractions has been questioned (Wander 2004). Extraction procedures based on the reactivity of SOM can affect stabilization mechanisms that are of different relevance in different soils (von Lützow et al. 2007). Physical (size, density) soil fractionation techniques have the advantage of recovering relatively unaltered primary and secondary organo-mineral complexes (Christensen 1996). Physical fractionation techniques designed to isolate soil aggregates (e.g. Elliott 1986; Six et al. 2002) usually obtain different size fractions of aggregates from one horizon, but the original pore structure, aggregate hierarchy and organisation are not preserved. When studying SOM, however, this pore structure can be relevant, e.g. the behavior of similar materials with different c/f related distributions or different structures after fractionation (Stoops 2003) (Fig. 2.1). Recent research has shown that the accessibility of SOM, rather than its chemical characteristics, is the major factor governing SOM turnover in the soil (Dungait et al. 2012).

Few researchers have studied SOM in its original structure and its relation to soil porosity and pedality at detailed scales (Descheemaeker et al. 2009; Kooijman et al. 2009) and soil micromorphology is available to characterize SOM variability at the scale of materials. The spatial variability of SOM at a nanoscale, however, is being satisfactorily developed in synchrotron facilities, which improve the precision of measurements (X-ray, IR) and produces SOM maps of microaggregates (Lehmann and Solomon 2010).

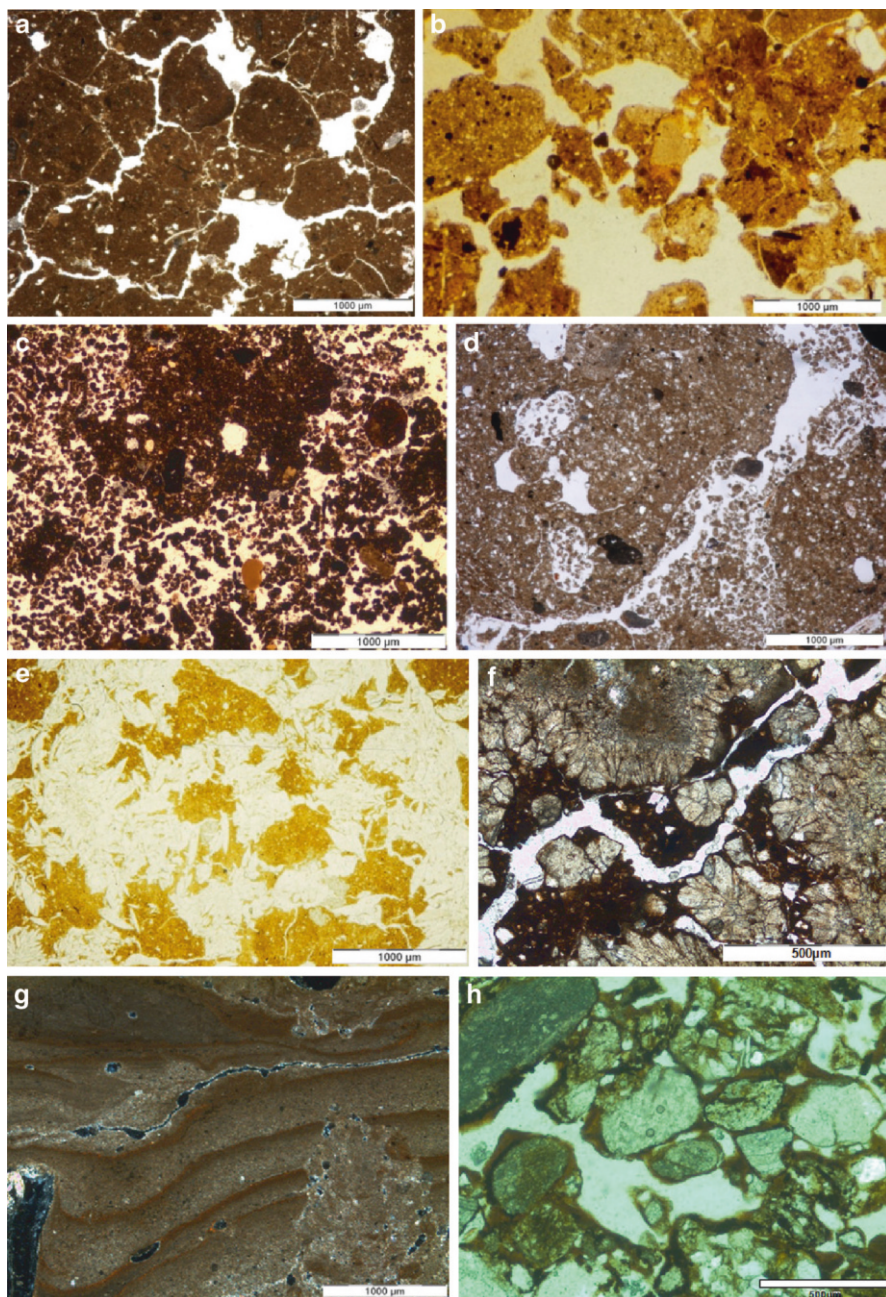


Fig. 2.1 Examples of soil fabrics and the meaning of the associated aggregates produced by physical fractionation. Only microphotograph (g) is under XPL, the rest is under PPL. (a) and (b) Classical well-separated microstructure. Its physical fractionation produces aggregates with a fairly straightforward interpretation. (c) and (d) Hierarchical and juxtaposed structures. Their physical fractionation produces aggregates with different functionalities regarding C storage (inside the pore infillings/in the groundmass). (e) and (f) Structures produced by fissuration caused by gypsum and calcite accumulation respectively. The aggregates produced by a physical fractionation have a very different meaning than in (a) and (b). (g) and (h) Laminated fabrics (g) or chitonic g/f distributions (h) warrant the study of SOM distribution; these fabrics yield meaningless results through physical fractionation

The objective of this chapter is to review the potential and limitations of classical micromorphology in the study of SOM, to explain why we think it has not been more widely used when studying SOM in relation to porosity and microsites, and to make some recommendations in order to fill in this methodological gap in the continuum of observation from landscape to submicroscopy.

Optical Microscopy of SOM

Micromorphology of SOM is largely influenced by Babel's work, whose "Micromorphology of Soil Organic Matter" (Babel 1975) has been the basis for the current system of soil thin section description (Bullock et al. 1985; Stoops 2003). He presented a comprehensive review of the knowledge of SOM micromorphology and its relation to SOM chemical composition and biology including information about fabric, structure, position related to pores and minerals, genesis and evolution.

The works of Babel and of other micromorphologists (e.g. Bal 1973; Fox and Parent 1993; Kooistra 1991) address the basis of some of the SOM sequestration principles (e.g. degree of decomposition, processes of incorporation into the soil matrix, protection in aggregates), therefore they should be considered in present-day soil carbon studies. The most recent reviews on SOM (Stockmann et al. 2013), soil biochemistry (Totsche et al. 2010), SOM micromorphology (Stolt and Lindbo 2010), and some studies on SOM dynamics (e.g. Nannipieri and Eldor 2009) stress the need of properly addressing the relationship between SOM and the soil (micro)structure, which would assist in understanding SOM and CO₂ responses to global changes.

The guidelines by Stoops (2003) classify SOM according to the extent to which its origin can be recognized. This can yield information about the position, fabric, and degree of decomposition of SOM higher than microscope resolution (about 20 µm), the general composition (origin of organic residues, presence of some components like phlobaphene, cellulose, lignine), the spatial relation with structure and mineral components; quantitative data by image analyses, and therefore the frame or location for further investigations. SOM micromorphology gives no information about detailed composition of SOM, nor about SOM particles smaller than the microscope resolution and the thickness of a thin section (about 20 µm). Other problems using classical micromorphology for the study of SOM are found in Table 2.1. Although most of the information is descriptive, SOM quantification for particulate organic matter is also possible through point counting (e.g. Davidson 2002) or image analyses (Poch and Antúnez 2010; Marks et al. 2012).

Fluorescence Microscopy

Babel (1972, 1997) used fluorescence microscopy through UV transmitted light for the study of organic matter, because some organic components (cell walls of fungi hyphae) are autofluorescent, whereas colloidal organic matter, such as found in

Table 2.1 Problems and their solutions when studying SOM with the optical microscope

Problem	Solution
Distinguishing SOM from other opaque soil components	Staining or dissolution techniques; fluorescence microscopy (references in Stolt and Lindbo 2010)
Identifying different types of particulate organic materials	Combinations of different images obtained with optical and fluorescence microscopy (e.g. biochar identification; Marks et al. 2012)
Distinguishing organic impregnations from other soil components or the soil matrix e.g. Fe or Mn compounds	Thin section pre-treatments with acids (selective dissolution; Bullock et al. 1975)
Drying of samples creating artifact cracks	Acetone or dioxane drying; alternative drying (references in Stolt and Lindbo 2010)
Sampling difficulties due to lack of coherence of organic matter	Encasing techniques: plaster, polymers (see Murphy 1986; Benyarku and Stoops 2005)
Inferring 3D features from 2D images	Binocular observations, adequate number of replicates, making vertical and horizontal sections, use of Computer Tomography Imaging
Lack of information about micromorphology of soil organic components	Build-up knowledge starting from existing SOM micromorphology

spodic horizons, is not. Special stains may be used to cause specific secondary fluorescence in components such as plant tissue or bacteria (Altemüller and Van Vliet-Lanoë 1990; Bruneau et al. 2005). The resulting images provide information about the type and distribution of organic matter and soil biota in relation to the mineral, aggregate and soil processes such as migration of organic matter or clays (Eickhorst and Tippkötter 2008; Tippkötter et al. 1986; Hall 1996; Wierzechos and Ascaso 1998; Driese and McKay 2004).

SEM and Microanalyses

Scanning Electron Microscopy (SEM) and the associated Energy Dispersive X-Ray (EDX) analysis is the first submicroscopic technique to provide maps of element distribution, and relative atomic mass when viewed through Back Scattering Electron Mode. Its application to the study of SOM is indirect since the detection of SOM may be masked by the impregnating resin and also because of the ubiquity of its elemental components (C, H, O) in soils. The technique has been used to trace specific elements in the soil-root interphase (Adamo et al. 1998; van Breemen et al. 2000), and in polluted or mined soils where SOM can be marked by a specific element (Tomlin et al. 1993; Arocena et al. 2010; Sort and Alcañiz 2001). Some others (e.g. Miltner et al. 2011) have used SEM-EDX micrographs to identify and differentiate organic components (e.g. microbial cells) from other soil elements.

NanoSIMS

Nano-scale Secondary Ion Mass Spectrometry (NanoSIMS) consists of a combination of high-resolution microscopy and isotopic probing, providing spatially-referred information on the isotopic composition of materials. It can be used to produce high-definition 2D maps (some nm) of isotopic abundance in the upper layers of a soil sample. Initial studies (Herrmann et al. 2007a, b) focused on the location and activity of microorganisms in the soil matrix using ^{15}N labeling of SOM, with the aim of characterizing the biophysical interface in soils. Recent studies using isotopic labeling (^{13}C and/or ^{15}N) of substrates added to the soil (Mueller et al. 2012), observed the distribution of fresh SOM (particulate and dissolved) in soil aggregates. Advances in sample preparation (avoiding embedding and therefore the interferences of resin in the analysis), have added to the potential of this technique to identify sorption sites of SOM (Heister et al. 2012), and organo-mineral associations (Hatton et al. 2012). These authors have also combined NanoSIMS with SEM, and Elemental Analysis and Isotope Ratio Mass Spectrometry (EA-IRMS) on soil fractions in a multi-scale approach that allows for some quantification and a better identification of SOM forms within the soil matrix. Remusat et al. (2012) have shown how the combination of NanoSIMS, Scanning Transmission X-ray Microscopy (STXM) and Near Edge X-ray Absorption Fine Structure Spectroscopy (NEXAFS) allowed relating the presence of metabolites in intact soil aggregates, and indicates the potential of this technique for in-situ SOM analysis. As outlined above, classical SEM and micromorphological studies are however still necessary in combination with this new technology for an accurate assessment of SOM distribution in the soil and its relationship to ecological processes and SOM stabilization.

DRIFT-FTIR

Fourier Transformed Infrared Spectroscopy (FTIR) is an Absorption Spectroscopy where the sample is subjected to a range of wavelengths in the IR, and the obtained interferograms are decomposed by Fourier transforms. Its application to surfaces is called DRIFT: Diffuse Reflectance Infrared Fourier Transform Spectroscopy, whereby areas occupied by specific functional groups of a given element can be located on a surface. One of the first applications of FTIR to SOM studies was point analysis of C functional groups on intact aggregates and soil peels (Arocena et al. 1995). Examples of SOM types mapping through FTIR and DRIFT can be found in Ellerbrock and Gerke (2004), Ellerbrock et al. (2010) and Leue et al. (2010), where it is concluded that differences of meso- and microsites in soils, as those affected by faunal activity or other structural mechanisms result in different SOM qualities and properties.

Synchrotron-Based NEXAFS and FTIR

Synchrotron-assisted imaging through NEXAFS and FTIR (Lehmann and Solomon 2010) is a promising technique that allows a detailed mapping of soil carbon (Kinyangi et al. 2006) and sulphur (Prietz et al. 2009) organic chemistry at a nanoscale. The main problems are the preparation of 200–800 nm thick sections, and the analysis of the results through PCA and background-effect removal, dependent on sample thickness and preparation. Upscaling is another issue.

Challenges and Recommendations

Micromorphology can give information on SOM location and quantification that cannot be obtained with other techniques. Some of the main difficulties arise from deriving 3D from 2D observations, and, in some cases, from the lack of representativeness. This can be overcome by a sound sampling from detailed field observations, and by an appropriate number of replications. The relationship to soil functioning has also to be taken into consideration, especially water movement, since all techniques use dried samples. It implies that in some cases specific drying techniques need to be applied in order to avoid artifacts. The combination of micromorphology with recent techniques for the SOM analyses of micro- and nanosites is promising, since it can give answer to functional relationships among SOM components and between SOM and the mineral phases of the soil.

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