Chapter 13

Methods for Studying Soil Organic Matter: Nature, Dynamics, Spatial Accessibility, and Interactions with Minerals

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TABLE S13.1 Chemical fractionation methods

Procedure	Reagent	Fraction targeted	Extracted compounds	Abundance of fraction (% TOC)	Assumed turnover or stabilization process	Uniform residence time?	Assumption verified?
Extraction	Cold water or dilute saline solutions	Dissolved organic matter	Sugars, amino-acids, phenols, . . .	0.2–2%	Extracted fraction is labile	No	No
	Alkali solutions (NaOH 0.1–0.5 M)	Humic substance extracts	Mixture of biogenic compounds (carbohydrates, proteins, lipids, . . .)	30–80%	Solubilized fraction is stable	No	No
	Organic solvents	Lipids	Lipids	2–6%	–	No	–
Hydrolysis	Hot water		Carbohydrates, amino-acids	1–5%	Extracted fraction is labile, management sensitive		Yes
	Concentrated acids (6 N HCl, 1 M H_2SO_4)		Carbohydrates, proteins, fatty acids	30–80%	Resistant fraction is chemically recalcitrant	No	Yes, fraction is millennia older than SOM

Oxidation	$KMnO_4$			10–30%	Extracted fraction is labile, management sensitive	?	?
	H_2O_2		Wide range, except natural waxes and pyrogenic compounds	20–93%	Resistant fraction is chemically recalcitrant. or protected in micropores. or by adsorption	No	Yes, fraction is millennia older than SOM
	$Na_2S_2O_8$		Wide range, except long chain aliphatics	16–99%	Resistant fraction is chemically recalcitrant or protected in micropores or by adsorption	?	?
	NaOCl		Wide range but alkyl C and pyrogenic compounds	26–96%	Resistant fraction is chemically recalcitrant or protected in micropores or by adsorption	Yes	Yes, fraction is millennia older than SOM

TABLE S13.2 Visualization methods for soil organic matter

Category	Name	Principle and Radiation Used	Image	Resolution	Sample Preparation	Visualization of Organic Matter	Additional Information on Organic Matter
Light microscopy	Stereomicroscopy	Incident light	3–D	≈ 10 µm	None (fresh samples)	Shape of coarse organic objects or organisms, color	
	Light microscopy bright field	Transmitted light	2–D	< 1 µm	Thin sample, either a deposit on glass slide or thin section of sample embedded in resin	Shape, color of organic matter	
	Epifluorescence microscopy	Transmitted light at a given wavelength that excites fluorescence in the sample	2–D	0.2 µm	Thin sample, either a deposit on glass slide or thin section of sample embedded in resin	Fluorescent stains, biological stains, immuno-stains	Autofluorescence of organic matter (and not minerals)
	Confocal (laser) microscopy	Transmitted light at a given wavelength that excites fluorescence in the sample	3–D	0.16 µm	Fresh sample (hydrated), but staining required	Fluorescent stains: biological stains, immuno-stains	Autofluorescence of organic matter (and not minerals)

Scanning probe microscopy	Atomic force microscopy (AFM)	A cantilever scans the surface of the sample and reacts to forces between surface atoms of the sample and its tip	3–D	1 nm	No preparation, can be hydrated, but flat surface needed	Shape of organic molecules deposited on mineral surfaces	Gives also information on surface properties such as hydrophobicity
Electron microscopy	Scanning electron microscopy (SEM)	Electrons reflected by surface of the sample allow a 3-D image to be reconstructed	3–D	10 nm	Fresh (environmental SEM), fresh and frozen (cryoSEM), air dried (conventional SEM) (under vacuum)	Shape of organic matter	Back scattered electrons gives information on atomic number contrast, energy disperse x-ray spectroscopy on elemental composition
	Transmission electron microscopy (TEM)	Electrons that go through the sample. Image is issued from transmitted and diffracted electrons	2–D	0.2 nm	Thin deposit on a grid or ultrathin section of sample embedded in a resin (under vacuum)	Shape of organic matter, reaction to specific stains	Energy disperse X-ray spectroscopy gives information on elemental composition. Energy loss spectrum gives information on elemental composition and chemical bonding

Continued

TABLE S13.2 Visualization methods for soil organic matter—Cont'd

Category	Name	Principle and Radiation Used	Image	Resolution	Sample Preparation	Visualization of Organic Matter	Additional Information on Organic Matter
X-ray spectro microscopy	Soft X-ray spectro microscopy in the water window (TXM, STXM)	X-rays transmitted through the sample	3–D	30 nm	Thin samples, deposits or thin sections of sample embedded in resin or shock frozen	Shape of organic matter, elemental distribution maps	Information can be obtained on binding states of elements using X-ray absorption near-edge structures (XANES or NEXAFS)
Secondary ion mass spectrometry	nanoSIMS	Incident ions detach sample ions of opposite charge which are collected and analyzed	2–D	150 nm	Dry and flat sample (under vacuum)	Elemental and isotopic distribution maps	
X-ray computed tomography	X-ray μCT	X-rays crossing the samples are attenuated, depending on density and atomic number of material	3–D	1 μm	Moist sample, no preparation	3-D image of solid particles and voids	Not yet imaged except large particles (>100 μm)