Spatial complexity of soil organic matter forms at nanometre scales

JOHANNES LEHMANN[1]*, DAWIT SOLOMON[1], JAMES KINYANGI[1], LENA DATHE[1], SUE WIRICK[2] AND CHRIS JACOBSEN[2]

[1]Department of Crop and Soil Sciences, College of Agriculture and Life Sciences, Cornell University, Ithaca, New York 14853, USA
[2]Department of Physics and Astronomy, State University of New York at Stony Brook, Stony Brook, New York 11794, USA
*e-mail: CL273@cornell.edu

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Organic matter in soil has been suggested to be composed of a complex mixture of identifiable biopolymers[1] rather than a chemically complex humic material[2]. Despite the importance of the spatial arrangement of organic matter forms in soil[3], its characterization has been hampered by the lack of a method for analysis at fine scales. X-ray spectromicroscopy has enabled the identification of spatial variability of organic matter forms, but was limited to extracted soil particles[4] and individual micropores within aggregates[5,6]. Here, we use synchrotron-based near-edge X-ray spectromicroscopy[7] of thin sections of entire and intact free microaggregates[6] to demonstrate that on spatial scales below 50 nm resolution, highly variable yet identifiable organic matter forms, such as plant or microbial biopolymers, can be found in soils at distinct locations of the mineral assemblage. Organic carbon forms detected at this spatial scale had no similarity to organic carbon forms of total soil. In contrast, we find that organic carbon forms of total soil were remarkably similar between soils from several temperate and tropical forests with very distinct vegetation composition and soil mineralogy. Spatial information on soil organic matter forms at the scale provided here could help to identify processes of organic matter cycling in soil, such as carbon stability or sequestration and responses to a changing climate.

Since the first investigations of soil organic matter in the eighteenth century[8], its composition has mostly been considered to be chemically very complex and to consist of mixtures of large molecules and reassembled microbial and plant debris[2]. This paradigm is about to change on the basis of findings that organic matter in soil extracts can be explained by plant and microbial biopolymers and their degradation products rather than distinct so-called humic macromolecules[1]. Recently, the notion that the spatial organization of soil organic matter within its mineral assemblage holds the key to an understanding of processes controlling its quantity and quality as well as turnover has gained increasing attention[3]. Specific compounds bearing certain chemical functional groups may be responsible for binding to mineral surfaces[9] and form very stable organic carbon pools with long turnover times, whereas others may be present as particulate organic matter within pores of aggregates and can be rapidly mobilized for example by tillage[2]. Different physical location of organic compounds that vary in their molecular structure and therefore chemical recalcitrance will also exhibit differential response to global warming[10]. Such fine-scale differences in organic matter forms may be expressed on a scale of several nanometres, which poses a challenge to determining their chemical speciation. If indeed systematic differences exist between organic matter forms at different locations in the soil matrix, important ecological

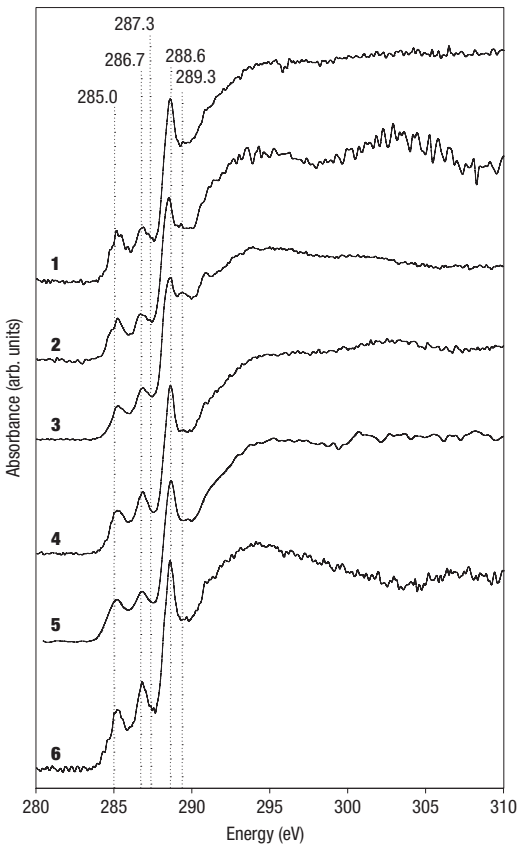

Figure 1 Molecular characterization of total soil carbon by C-1s NEXAFS spectroscopy using transmission spectroscopy of soil extracts obtained from different forest sites. 1, Arnot Forest on schist parent material in New York State, USA. 2, McGowen Forest on glacial till in New York State, USA. 3, Barro Colorado Island Forest on volcanic facies in Panama. 4, Embrapa Forest on Tertiary sediments in central Amazonia, Brazil. 5, Nandi Forest on biotite-gneiss in western Kenya. 6, Franz Josef Forest on schist outwash terraces in New Zealand.

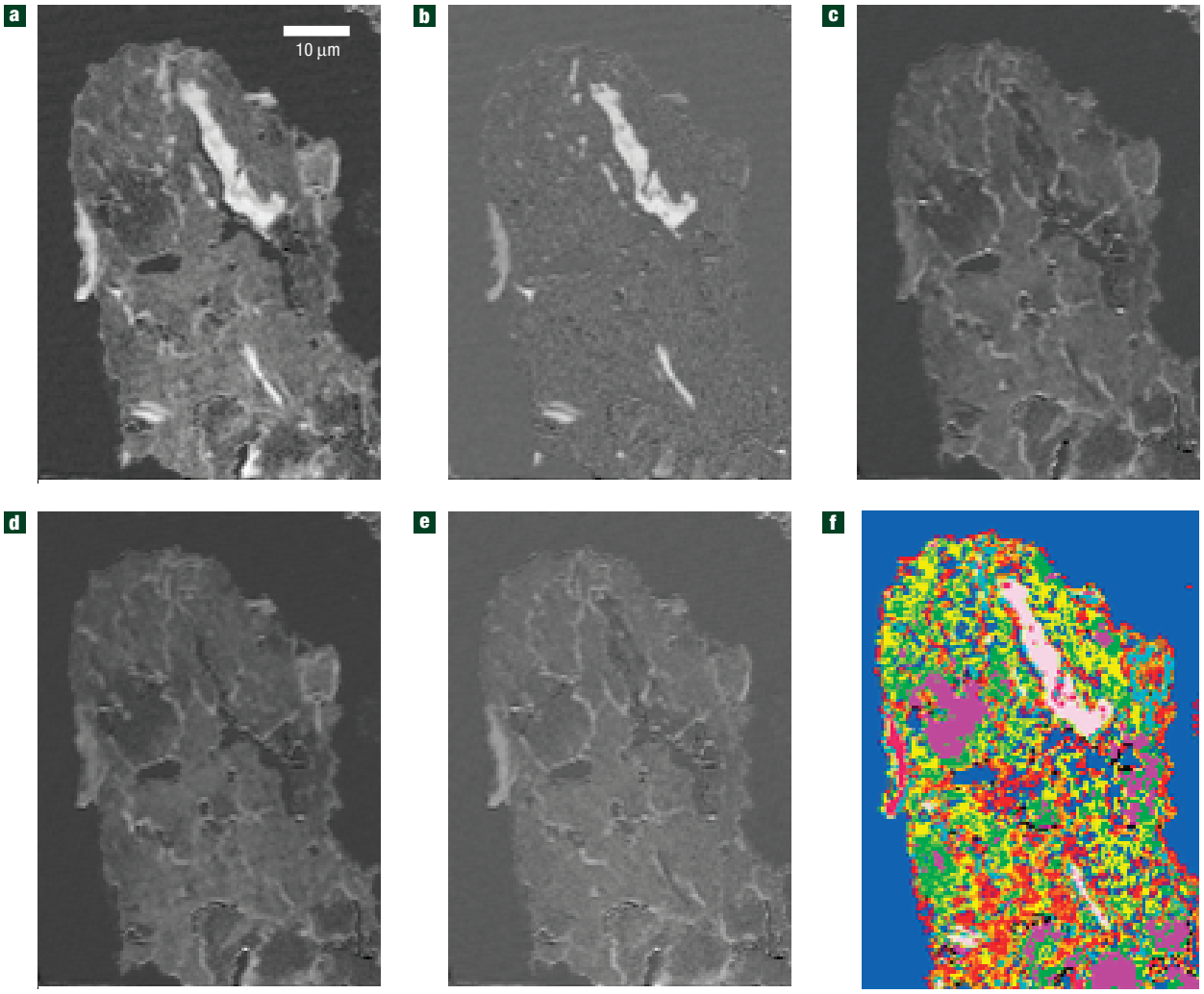

Figure 2 Distribution of carbon contents and molecular forms in a soil microassemblage from Nandi Forest (Kenya) determined by NEXAFS in combination with STXM. a, Total carbon (subtraction of energy region at 280–282 eV from 290–292 eV). **b**, Aromatic carbon (subtraction of energy region at 280–282 eV from 284–286 eV). **c**, Aliphatic carbon (subtraction of energy region at 284–286 eV from 287.3–287.8 eV). **d**, Carboxyl carbon (subtraction of energy region at 284–286 eV from 288–289 eV). **e**, Phenolic carbon (subtraction of energy region at 284–286 eV from 286.4–287.4 eV). **f**, Cluster map of carbon forms (clusters are shown separately with their spectra in Fig. 3).

information is lost by investigating only organic matter forms of the entire soil or not recognizing a specific spatial location of individual types of biopolymer.

The *in situ* investigation of fine-scale spatial heterogeneity of soil organic matter forms has mainly been hampered by the lack of appropriate analytical capabilities[4–6,11]. Near-edge X-ray fine structure (NEXAFS) spectroscopy provides a new view when coupled to scanning transmission X-ray microscopy[7,12] (STXM) and has offered valuable insight into organic carbon forms associated with particulate organic matter of marine origin[11], individual particles extracted from soil by water[4], black carbon particles[13,14] and individual pores within microaggregates[5,6]. However, the spatial arrangement of organic carbon forms across entire microaggregates has not been shown, which was obtained here by STXM in combination with soil preparation that preserves the spatial assemblage of the soil (see the Methods section).

Using NEXAFS spectroscopy, we found the organic carbon forms to be remarkably similar between acid or calcareous forest soil in North America, Panama, Brazil, Kenya or New Zealand (Fig. 1). These resembled spectral properties of organic matter extracted from forest soil in Germany[4] or clay fractions of Ethiopian forest soils[15]. Even organic matter extracts from soils at different stages of degradation have shown identical peak positions and comparable peak intensities of C (1s) NEXAFS spectra[16] which suggest similarity in organic carbon forms.

In stark contrast to the similarity in organic carbon forms between soil from very different environments, different spatially distinct regions within any given soil showed highly variable carbon functional group composition on scales of nano- and micrometres (Fig. 2; and Supplementary Information, Figs S1–S3). Spotty regions that were rich in aromatic carbon ring structures (284–286 eV, Fig. 2b) contrasted with a distribution of aliphatic carbon (287.3–287.8 eV, Fig. 2c) forming a network along pore structures. Similar to aliphatic carbon, regions rich in carboxylic/amide (288–289 eV, Fig. 2d) and phenolic/pyrimidine or imidazole carbon (286.4–287.4 eV, Fig. 2e) were found throughout the microstructure, yet with clear agglomeration. Compounds with corresponding organic carbon forms occupied

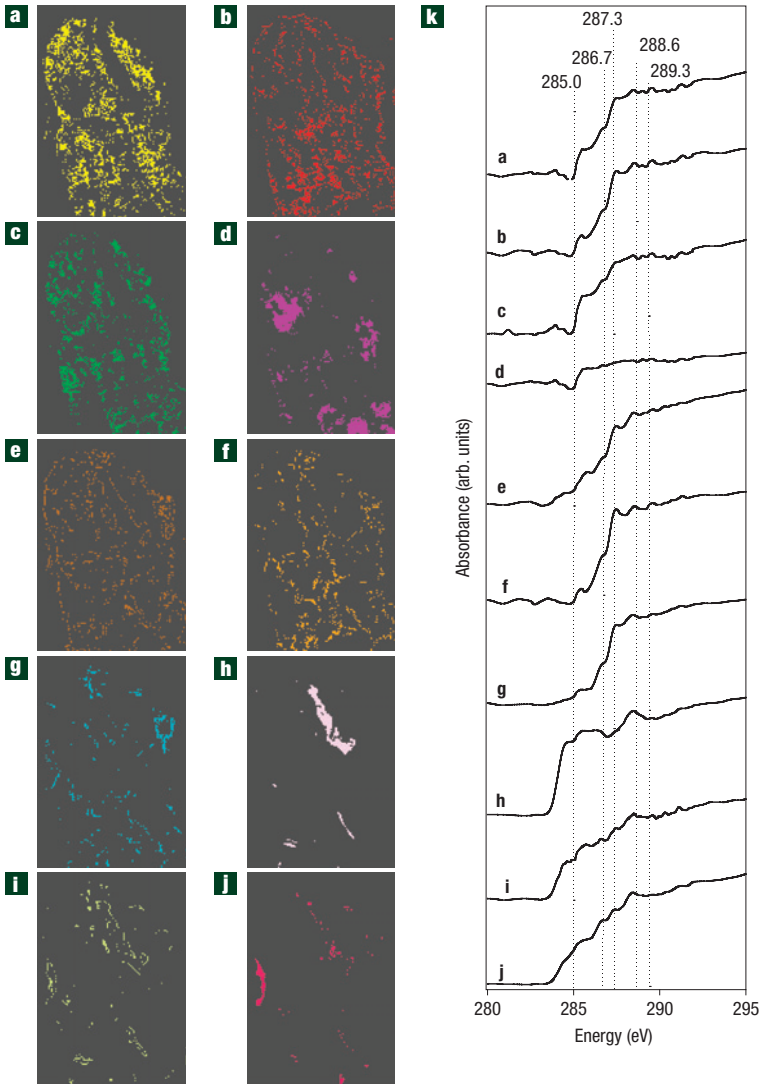

Figure 3 Characterization of organic carbon forms at different regions within a soil microassemblage from Nandi Forest (Kenya) determined by NEXAFS in combination with STXM. a–k, Spatial distribution of different clusters (**a–j**) and corresponding spectra (**k**) from principle component analysis.

distinct regions within the soil microassemblage as revealed by principle component and cluster analysis of the entire spectral properties (Fig. 2f). These spectral properties varied to a great extent either showing characteristic peaks indicative of phenolic, aliphatic and carboxylic/amide carbon (Fig. 3i,j), aliphatic and carboxylic/amide carbon (Fig. 3e–g) or aromatic carbon (Fig. 3h). The combination of peak positions and intensities suggests that several carbon-rich regions be identified as altered plant material (Fig. 3j, compare with Supplementary Information, Fig. S4), microorganisms and their metabolites (Fig. 3g, compare with Lawrence *et al.*[17] and Liang *et al.*[14]) or black carbon forms (Fig. 3h, compare with Lehmann *et al.*[13]). On a finer scale (50 nm resolution), these differences are even more pronounced and provide a clear indication for distinct organic matter forms such as microbial matter (see Supplementary Information, Figs S1,S2). With advances in X-ray focusing, a spatial resolution of 10 nm seems achievable in the near future.

Several pieces of evidence explain this extremely heterogeneous distribution of organic matter forms on a scale of nanometres. Microorganisms have shown a non-uniform and rather cluster-forming distribution in soil as a response to their habitat[18]. Despite the large number of about 10^{12} bacteria in 1 g of soil, only far less than 1% of the soil surface area is covered with microbes[3], as they inhabit only very small and distinct spaces in soil. Consequently, the area affected by microorganisms at any given time is small, and modifies the carbon forms in its immediate environment for example through the production of extracellular polysaccharides[19]. In addition, the soil aggregate and pore architecture may influence organic matter distribution. For example, organic matter filling micrometre-size pores within aggregates was found to be chemically distinct from coatings of mineral domains[5]. Different forms of organic matter may bind to different mineral surfaces[9] and some minerals such as short-range-order minerals are able to bind more organic material than others[20]. Finally, the chemical persistence of organic matter entering the soil carbon pool may vary to a large extent, yielding spatially distinct regions of also chemically dissimilar organic matter forms such as in the case of particulate black carbon (Fig. 4c), which can remain in soils for very long periods of time[21].

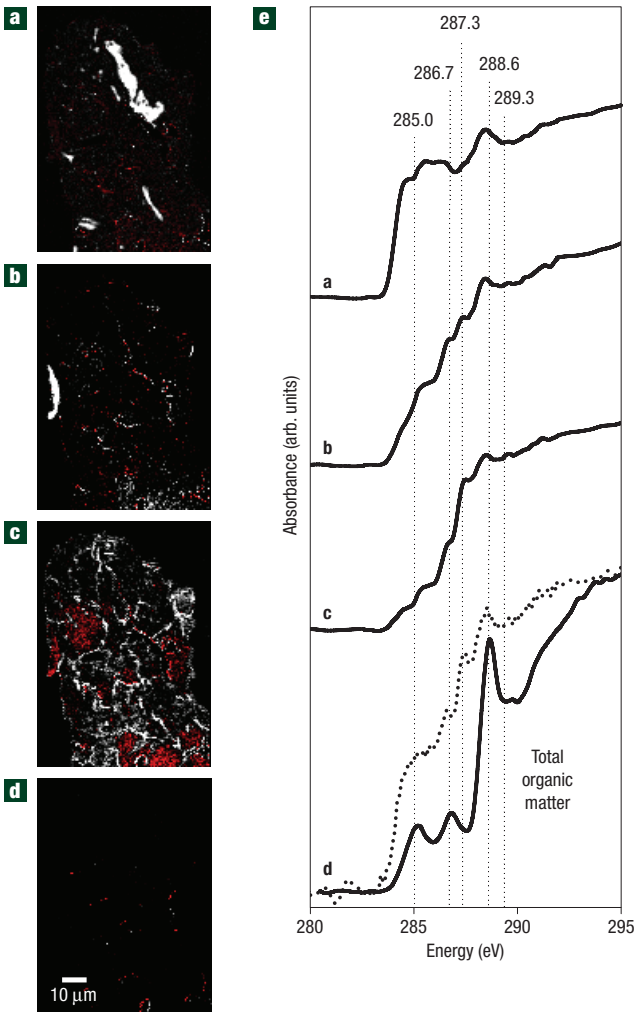

Figure 4 Identification of regions with spectral properties of total organic carbon within a soil microassemblage from Nandi Forest (Kenya) determined by NEXAFS in combination with STXM. a–c, Target maps from clusters obtained by principle component analysis. **d**, Target map of total organic carbon. **e**, Corresponding spectra (the fit for total organic carbon is indicated by a dotted line). White regions in the target maps indicate areas that are well characterized by the corresponding target spectrum.

In addition to the fact that the organic matter forms of contrasting regions were significantly different on a very fine spatial scale, none of these regions was similar to the organic matter of the entire soil (Figs 3 and 4d, Supplementary Information, Fig. S2). This interpretation was not hampered by the fact that the STXM analyses were done on isolated aggregates, as results from separated density fractions using fluorescence yield for NEXAFS revealed near-identical spectral features compared to the total soil with only minor shifts in peak intensities (see Supplementary Information, Figs S5,S6). In a reverse approach, the presence and location of total organic matter forms (extracts were a valid proxy for total soil organic matter as demonstrated by Supplementary Information, Fig. S6) were tested by singular value decomposition. The absence of intensely bright areas which would indicate the presence of the target spectrum (in this case the total organic matter) and the poor fit (Fig. 4d) confirm the assertion that organic matter forms of the entire soil can on a very fine scale not be discerned in any region of the investigated soil. Organic matter in soils may to a significant extent consist of identifiable and distinct molecular forms such as microbial and plant biopolymers not only in complex mixture[1] but in very complex spatial arrangements which as a sum correspond to total organic matter but individually are very different from the molecular characteristics of total organic matter.

The implications of this fine spatial distinction between individual regions and total organic matter can be easily understood when looking at the contrasting properties of black carbon and microbial organic matter. Black carbon is the residue of incomplete combustion of organic matter[21] and possesses entirely different spectral properties compared with microbial organic matter as seen from our NEXAFS studies (compare Fig. 3g,h and Fig. 4a,c indicative of microbial organic matter and black carbon, respectively; for higher-resolution observation, see Supplementary Information, Fig. S2d,e). Microbial carbon is characterized by amide, carboxylic and aliphatic carbon forms with dominant peaks between 287 and 289 eV (refs 14,17), whereas black carbon is dominated by aromatic carbon with a prominent peak at 285 eV (ref. 13). Black carbon is spatially well separable on a fine scale, because it mainly exists as discrete particles, whereas microbial products are embedded in pore spaces and bound to mineral surfaces (Figs 3 and 4). Therefore, as an example, areas in soil characterized by black carbon will exhibit entirely different properties such as decomposability than those occupied by microbial organic matter. In addition, interactions with different minerals will depend on co-location and forms of organic matter[9] with implications for its stability because soil mineralogy can significantly alter the turnover time of soil organic matter[20]. A composite analysis will mask the resolution of these interactions between organic matter and minerals or microorganisms. This consideration does not only apply to black carbon but also to other organic matter on various scales in the studied soils and assemblages shown here, which seem to occupy distinct spaces and have vastly differing organic matter forms.

This assessment of the nanoscale distribution of organic matter forms in soil does not render characterization of total organic matter redundant. But it clearly shows the limitation of integrated observations of a spatially complex mixture with very distinct properties on a very fine scale as found in soils. An interpretation of organic matter changes as a reaction to broad shifts in environmental conditions (that is, vegetation, temperature, management, soil type) is very useful as a starting point for formulating hypotheses, but may benefit from spatial information similar to that shown here for identifying processes of organic matter cycling in soil, such as carbon stability, sequestration and responses to a changing climate.

METHODS

Soil samples were collected from old-growth forests at Arnot on glacial till, forming acid Dystrochrepts (42° 16′ N, 76° 28′ W); at McGowen on glacial lake deposits, forming Dystrochrepts with pH 6 (in water) (42° 26′44″ N and 76° 27′2″ W) both in New York State, USA; at Barro Colorado Island on volcanic Caimito formation in Panama with Alfisols[22] (9° 9′ N, 79° 51′ W); at the Embrapa experimental station near Manaus, Brazil, on Tertiary sediments, forming Hapludoxes[23] (02° 54′02″ S and 59° 58′55″ W); in the Nandi area on biotite-gneiss in western Kenya, forming Hapludoxes[5] (00° 04′30″ N and 34° 58′34″ E); at the Franz Josef glacier on schist outwash in New Zealand[24] (43°28′ S and 169° 55′ E).

Organic matter forms in each soil were determined by C (1s) NEXAFS spectroscopy[7]. Measurements were done on total soil and density fractions of the Kenyan soil, total organic matter extracts or intact sections of the organomineral assemblage. The total soil, density fractions and organic matter extracts were analysed using a fluorescence detector at the SGM beamline of the Canadian Light Source. Intact soil microaggregate assemblages and organic matter extracts were analysed on a scanning transmission X-ray microscope at the X1A1 beamline of the National Synchrotron Light Source.

Organic matter was extracted from the soils with a mixture of 0.1 M NaOH and 0.4 M NaF (ref. 16). The soil from the Nandi Forest was fractionated by density using NaI, which yielded a free light fraction with a density below 1.8 g cm^{-3}, an intra-aggregate light fraction and an organomineral fraction[25]. The density fractions, the total soil and the freeze-dried organic matter extracts were pressed into indium foil (1 mm thickness) and measured against a sample-free and scraped indium blank under vacuum from 280 to 310 eV across the carbon K-edge at the SGM beamline. The step size was 0.5 eV from 280 to 282.5 eV, 0.1 eV from 282.5 to 292 eV, 0.5 eV from 292 to 305 eV and 1 eV from 305 to 310 eV. The dwell time was 200 ms and the exit slit was 50 μm. Results were calculated against a reference measurement[26], background corrected, normalized and smoothed once by using Athena[27]. Energy calibration was done with CO to 287.38 eV.

Free stable microaggregates (20–250 μm diameter) were isolated from soils and sectioned to a thickness of 200–400 nm by hydration and subsequent shock-freezing using a cryo-microtome[6]. This procedure enabled the spatial arrangement of organic matter in soils to be observed at high resolution without compromising the spatial integrity of the organomineral assemblage. Common embedding media such as resins cannot be used as they contain organic carbon forms[13]. Samples were mounted on silicon-monoxide-backed Cu transmission electron microscopy grids (200 mesh, no. 53002, Ladd Research) and measured in transmission under helium atmosphere[7]. A STACK data set was collected by imaging in *X* and *Y* dimensions, then changing the monochromator by energy increments of 0.3 eV (dwell time 1 ms) for the energy range from 280 to 282.5 eV, of 0.1 eV up to 292 eV (dwell time 2 ms), of 0.5 eV up to 305 eV (dwell time 3 ms) and of 1.0 eV up to 310 eV (dwell time 4 ms). The microscope focuses X-rays using a zone plate to a focal point of 40 nm. Entire aggregates were scanned using a spatial resolution of 500 nm with high-resolution scans of 50 nm in smaller regions within aggregates.

Individual images scanned across all energy levels were stacked (Stack-Analyze 2.6 software, C. Jacobsen, SUNY Stony Brook), then aligned in *X* and *Y* using cross-correlation (with 290 eV as a reference). Optical density maps of total carbon and carbon forms were obtained by subtracting spectral regions below the C K-edge at 280–282 eV from different regions above the C K-edge. For the spatial analyses, the STACK data were orthogonalized and noise-filtered by principal component analysis. Cluster analysis was used to identify sample regions with similar spectral properties and to identify regions for which target spectra of total organic matter were defined in comparison with cluster spectra (PCA_GUI 1.1.1)[28]. The first 2–4 components and 6–20 clusters were used on the basis of the eigenvalues, eigenimages and eigenspectra[29] calculated by the principal component analysis.

Spectra from the organic matter extracts were collected from three different spots through dried droplets mounted on Si_3N_4 windows ratioed to adjacent sample-free regions. The grating was scanned from 280 to 310 eV in energy steps of 0.1 eV with a dwell time of 120 ms. The sample *Z* stage was moved by 10 μm to defocus the beam spot from an area of 50 nm to an area approximately 1 μm in size[16]. The monochromator was calibrated before each session using the 3*s* absorption band of CO_2 at 292.74 eV.

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Correspondence and requests for materials should be addressed to J.L.
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Author contributions

J.L. planned the study, conducted the X-ray measurements, analysed the data and developed the paper. D.S., J.K. and L.D. prepared the samples and provided input to the manuscript. S.W. and C.J. aided in X-ray and data analyses and commented on the manuscript.