Organic Carbon Distribution, Speciation, and Elemental Correlations within Soil Microaggregates: Applications of STXM and NEXAFS Spectroscopy

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Revised version, July 10th, 2007
ABSTRACT

Soils contain the largest inventory of organic carbon on the Earth’s surface. Therefore, it is important to understand how soil organic carbon (SOC) is distributed in soils. This study directly measured SOC distributions within soil microaggregates and its associations with major soil elements from three soil groups (Phaeozem, Cambisol, and Ultisol), using scanning transmission X-ray microscopy (STXM) and near-edge X-ray absorption fine structure (NEXAFS) spectroscopy at a spatial resolution of 30 nm. Unlike previous studies, small intact soil microaggregates were examined directly in order to avoid preparatory procedures that might alter C speciation. We found that SOC exists as distinct particles (tens to hundreds of nm) and as ubiquitous thin coatings on clay minerals and iron-oxides coatings. The distinct SOC particles have higher fractions of aromatic C than the coatings. NEXAFS spectra of the C coatings within individual microaggregates were relatively similar. In the Phaeozem soil, the pervasive spectral features were those of phenolic and carboxylic C, while in the Cambisol soil the most common spectral feature was the carboxyl peak. The Ultisol soil displayed a diffuse distribution of aromatic, phenolic, and carboxylic C peaks over all surfaces. In general, a wide range of C functional groups coexist within individual microaggregates. In this work we were able to, for the first time, directly quantify the major mineral elemental (Si, Al, Ca, Fe, K, Ti) compositions simultaneously with C distribution and speciation at the nm to µm scale. These direct microscale measurements will help improve understanding on SOC-mineral associations in soil environments.
INTRODUCTION

Soil organic carbon (SOC) is the largest reservoir of organic carbon on Earth’s surface. Understanding factors controlling SOC stability is critical to predicting changes in the global C balance and its broader environmental consequences (Kwon and Schnoor, 1994; Lal, 2004; Lal et al., 2004). Humic substances and SOC have been studied extensively over the past century (Hedges and Oades, 1997; Huang and Schnitzer, 1986; Stevenson, 1994). Because humic substances contain disordered mixtures of a variety of functional groups (carboxyl, carbonyl, amino, phenol, and others) on aliphatic and aromatic linkages, they exhibit amphiphilic and amphoteric behavior. Although earlier studies led to depictions of very large (> 10 kDa) polymers, various lines of evidence now indicate that individual structures are smaller (0.1 to 1 kDa), and that they form into larger, transient micellar associations that can contain biomolecules (Sutton and Spisito, 2005). The long-term persistence of the refractory fraction of SOC is puzzling in macroscopically aerobic soils in view of the thermodynamic drive to oxidize C. Several factors are believed to contribute to SOC stabilization, including physical protection, association with mineral phases, and chemical recalcitrance (Baldoch and Skjemstad, 2000; Sollins et al., 1996). Given the fact that SOC is oxidized within much shorter times when directly accessible to microorganisms and their extracellular enzymes, it is inferred that physical protection from these agents by restrictive micropores is important in soils (Krull et al., 2003; Rovira and Greacen, 1957; Six et al., 2004). The recalcitrant SOC fraction has been found to be well correlated with the amorphous and poorly crystalline oxide (allophane, imogolite, ferrihydrite) fraction in soils (Mikutta et al., 2005; Torn et al., 1997).
Other studies show general correlations between SOC retention and the fine solids fraction (clay minerals and Fe-oxides) (Eusterhues et al., 2003; Eusterhues et al., 2005; Kaiser and Guggenberger, 2003). Over much longer time scales, intrinsic chemical recalcitrance appears to allow fractions of highly aromatic black carbon particles to resist oxidation for thousands of years (Krull et al., 2003; Lehmann et al., 2005; Pessenda et al., 2001).

The macroscopic scale at which most SOC measurements are made has prevented conclusive identification of dominant associations on specific minerals in natural soils. Even studies of very small quantities of soils remain effectively bulk analyses when SOC associations with diverse minerals are not determined at the scale of individual grains. Without obtaining such direct, grain-scale understanding obtained from natural samples, the mechanistic foundation for SOC-mineral stabilization will remain lacking. Recent microscopic and microspectroscopic analyses are beginning to address the issue of SOC-mineral associations (Benzerara et al., 2005; Chenu and Plante, 2006; Lehmann et al., 2005; Schumacher et al., 2005), and may eventually provide a basis for building more robust models of larger scale C cycling.

Advantages of soft X-ray methods (STXM and NEXAFS) over TEM-EELS for studies of C speciation have been demonstrated (Braun et al., 2005). Previous applications of the STXM to SOC have yielded analyses of C functional group speciation (Lehmann et al., 2005; Schumacher et al., 2005). However, most synchrotron beamlines optimized for soft X-ray spectromicroscopy for C speciation do not have sufficient flux at higher energies to quantify Al and Si, which are by far the most abundant mineral cations in soils. Using the Beamline 11.0.2 (Kilcoyne et al., 2003) at the Advanced Light Source (ALS), Lawrence Berkeley National Laboratory, this study was able to quantify the distribution of major elements in mineral particles simultaneously with determinations of spatial distributions of SOC and SOC functional groups at
the grain surface scale (tens of nm up to 10 µm) within soil microaggregates. This scale is the natural and relevant one at which to compare SOC partitioning to fine soil aggregates of different mineralogy. Another technical difficulty encountered in studies conducted at this scale is that of making thin sections (≤100 nm) of soil microaggregates. Soil microaggregates contain both extremely soft SOC and hard minerals, such that the fixing medium must imbibe into the nanopore networks of both SOC and minerals, then harden in order to permit microtome cutting (LEHMANN et al., 2005). Possible alteration of C speciation during preparation of thin sections remains to be determined. In this study we took a different approach that circumvented the need to fix, mill, or cut microaggregates. Soil microaggregates were simply air-dried, sieved, and analyzed with the STXM to obtain maps of SOC speciation and associations with major elements within individual mineral particles at a spatial resolution of 30 nm.

**EXPERIMENTAL**

**Soils and Sample Preparation**

Samples selected for this study come from three soils, well characterized in previous investigations on SOC. Some important bulk soil properties are summarized in Table 1, and more detailed characterization of bulk properties are provided in the references associated with each soil. The Phaeozem sample used in this experiment is from a German deciduous forest soil of basalt parent rock, with a clay fraction that is primarily smectite (27% clay, 4% silt, 24% sand) (KLEBER et al., 2005; SIREGAR et al., 2005). The bulk soil cation exchange capacity is 38 cmol\(_{c}\)kg\(^{-1}\), its specific surface area is 37 m\(^2\)g\(^{-1}\), and a pH value of 6.0 was measured from a soil water extract at a 1:1 soil to water ratio. The Cambisol sample is from a German coniferous
forest soil developed from gneiss (KLEBER et al., 2005), with vermiculite, kaolinite, and illite as dominant clay minerals (30% clay, 30% silt, 40% sand), and is the most acidic soil (pH 4.2) included in this study. The soil sample has a cation exchange capacity of 75 cmol,kg⁻¹, and a specific surface area of 23.0 m²g⁻¹. An Ultisol sample was obtained from a temperate forest soil in Tennessee (USA) formed on shale saprolite. This soil was from the Background Site used by researchers in the U.S. Department of Energy’s Natural and Accelerated Bioremediation Research Program (www.esd.ornl.gov/nabirfc). After sieving (2 mm), the sample contained 12% clay (primarily illite), and 43% and 45% silt and sand, respectively. The bulk soil cation exchange capacity is 30 cmol,kg⁻¹, and the pH of its soil water extract is 6.2.

The < 2 mm soil particles were further sieved to remove the greater than 43 µm fraction. To minimize the disturbance imposed on particle assemblages used for microspectroscopic analyses, no further treatments were applied to the < 43 µm soil particles. Soil particles were supported on silicon nitride (Si₃N₄) windows (1 mm², 200 nm thick). The air-dried and sieved particles were deposited onto Si₃N₄ windows (no chemicals used), then gently shaken to remove the largest particles (typically > 10 µm, based on sizes of microaggregates remaining during SXTM measurements), and the Si₃N₄ window was placed on the STXM stage. Although this procedure does not preserve spatial relations among large numbers of individual mineral grains (original macroaggregate structure), it does retain small microaggregates that are themselves stable associations of colloid-size particles including natural nanoparticles. This procedure has the advantage of avoiding exposure of samples to potentially harsh physicochemical disturbances. Thus, elemental associations and chemical speciation within individual particles and small assemblages of particles remain practically undisturbed.
The scanning transmission X-ray microscope (STXM) is a synchrotron-based, soft X-ray microspectroscopic instrument that permits chemical mapping of thin specimens. In order to obtain maps of major mineral forming elements along with C maps, STXM measurements in this study were obtained on the Molecular Environmental Sciences Beamline 11.0.2 (KILCOYNE et al., 2003) at the Advanced Light Source (ALS), Lawrence Berkeley National Laboratory. Photons are admitted to the STXM chamber through a Si$_3$N$_4$ window and focused onto the sample by a zone plate with a working distance of 0.5 to 9 mm, depending on photon energy. The available energy range on Beamline 11.0.2 is 90-2150 eV. This unusually wide STXM energy range is essential for understanding C associations with soil particles because it allows quantification of all major mineral cations (most notably Al and Si). An order-selecting aperture is placed between the zone plate and the sample. The sample is scanned relative to the focused X-ray beam, and the transmitted intensity is recorded as a function of sample position using a scintillator with photomultiplier tube or a photodiode. The zone plate can focus the beam down to a spot size of 30 to 50 nm. The smallest resolvable features are about 25 nm. Several different zone plates are used depending on photon energy, required spatial resolution and minimum working distance. The maximum energy resolution E/ΔE is better than 7500, and a resolving power better than 3000 is achieved at a photon flux of $10^8$-$10^9$ per second in the sub-50 nm focused beam at energies of about 200-1,600 eV. The maximum raster scan range is 20x4 mm, while the minimum step size is 2.5 nm. Images with up to 3000 x 2000 pixels can be acquired at any spatial size regime meeting these limits. The very wide dynamic range of the spatial scanning system, achieved with continuous interferometric feedback of positioning with an accuracy of 5 nm, is a very powerful aspect of this STXM.
Regions on the Si₃N₄ window supporting particles of suitable size were located under low magnification (e.g., 50 to 500 µm field of view, 500 nm pixels, 0.5 ms dwell time) during initial scanning on the STXM with the monochromatic beam set at 288 eV (just above the C K-edge electron binding energy). The optical density \[ \text{OD} = \log(I_o/I), \] where \( I_o \) is the background photon flux transmitted through the Si₃N₄ window measured within a clean area on the same Si₃N₄ window, and \( I \) is the photon flux transmitted through a region within the sample] was used as an initial screening criterion for selecting particles. Regions with \( \text{OD} > 2 \) were too thick to obtain C maps. In general, microaggregates with lateral dimensions greater than several µm were usually too thick to permit significant transmission of soft (low energy) X-rays, thus were too thick to obtain quantitative elemental maps and chemical speciation. Using this constraint, small areas (typically 20 to 70 µm²) were selected on the Si₃N₄ windows containing particles having suitable thickness for detailed mapping and microspectroscopic measurements. The possible scale (size) dependence of SOC speciation and SOC:mineral mass ratios is beyond the scope of this study, but is important in relating bulk soil properties to these measurements. Particles were then scanned at higher magnification over a series of closely spaced energy steps using stack mode (JACOBSEN et al., 2000) to map K edge absorption spectra for C, over the energy range of 280 to 300 eV. This energy range was subdivided in order to collect spectra with smaller energy steps (0.10 eV) over the region associated with the main resonance peaks (284.0 to 289.0 eV), and larger steps (0.25 to 0.30 eV) elsewhere. These stack measurements for C NEXAFS were obtained with dwell times of 2 ms or less, and with pixel sizes from 40 to 80 nm. Maps of total C distributions were then obtained using a pixel size of 25 nm, as differences of 2 absorption images recorded with monochromatic X-ray energies of 280 and 288.4 eV for background and C absorption, respectively. The absorption images were converted to OD images before
subtraction. After obtaining C spectra and maps, STXM maps of other elements were collected at the same pixel size. Because a main objective of this study was to identify associations of SOC with specific soil solids, K-edge OD maps of Al and Si, and L-edge OD maps of K, Ca, and Fe were obtained over the same areas that C spectra and maps were collected. In addition, L-edge maps of Ti were collected to test the hypothesis that this element would occur primarily as highly localized TiO$_2$ particles. These maps were obtained by subtracting below-edge OD maps for each element from its corresponding above-edge OD map. Carbon NEXAFS stack alignment and analyses, and alignment of background and edge maps of other elements were done using aXis2000 (Hitchcock, 2006). In addition, individual elemental maps were aligned in aXis2000 by assigning common coordinates to distinct image features. Photon energies and other parameters used for elemental mapping are listed in Table 2. For determining carbon speciation, functional groups, resonance peak energy ranges, electron transitions, and literature sources are listed in Table 4.

RESULTS AND DISCUSSION

**Elemental Maps**

Maps of elemental distributions within microaggregates from the Phaeozem, Cambisol, and Ultisol are shown in Figure 1. The overall morphology of each microaggregate is shown in the upper left panel for each sample. These black and white STXM images are OD maps obtained at 710 eV, just above the Fe L$_{\text{III}}$ edge. The rainbow scale color maps represent distributions of relative elemental concentration (mass per unit area), with lowest values in dark
purple (black = zero), and highest values from each data set in red. The OD values from which these maps were derived are presented in the following section. While the elemental maps are presented in order of increasing atomic number (Z), the X-ray energies used for these maps are not progressively increasing with Z because both K- and L-edges were used (Table 2). These images show considerable chemical heterogeneity within small microaggregates, and individual particles of different chemical compositions are often visible with characteristic sizes in the 100 nm to 1 µm range (30 nm resolution). Because the lowest energies used for mapping are for C, and because heavier elements in minerals efficiently absorb X-rays in many regions of these maps, C is not quantitatively mapped over all regions. Regions that have relatively thick mineral particles will appear devoid of C simply because of insufficient X-ray transmission at the C K-edge. The two Cambisol particles with greatest Al and Si thickness only show their outlines in the C map; most likely because C coatings are only detectable along perimeters and not visible on bulk surfaces of thick, strongly absorbing grains. Despite this limitation, the maps from these samples each show broadly dispersed and locally concentrated (thick) distributions of C within soil microaggregates, consistent with earlier findings (CHENU and PLANTE, 2006). One unique and important advantage utilized in the present ALS 11.0.2 STXM-based study is that C distributions can be mapped along with major mineral elements.

Maps of major mineral elements Al, Si, K, Ca, and Fe, and the minor element Ti in the three samples are also shown in Figure 1. In all of these samples Ti occurs independent of other metals, primarily as very sparsely distributed sub-micron particles, consistent with refractory rutile or anatase (both TiO₂). The prevalence of Al, Si, and Fe, particularly Al and Si, in these maps is consistent with the fact that these elements are most common in soils, especially in the
clay size fraction (< 2 µm) dominated by aluminosilicate clays and Fe-(hydr)oxides. The elemental correlations of these image maps will be discussed in the following sections.

Correlations Between Elements

The extent to which C and other elements are spatially correlated was examined by aligning each elemental OD map to common reference features using aXis2000, then comparing OD values on a pixel-by-pixel basis to determine correlation coefficients among different elements. Plots of some elemental correlations are shown in Figures 2-4, and correlation coefficients between all elemental OD pairs are provided in Tables 3a-c. It should be kept in mind that these correlations are relative to the pixel scale, which is nominally 30 nm by 30 nm along the mapping plane, and highly variable in thickness. As noted previously, thicknesses at most locations shown are less than 100 nm, and the few thicker regions are prohibitively absorbing to map elements other than Al and Si. In the elemental correlation plots for C with Al, Si, and Fe, axes for estimated thickness of SOC, Al₂O₃, SiO₂, and Fe₂O₃ have been included to indicate approximate thicknesses of individual components. These thickness estimates were obtained by dividing OD values by the product RE·µ·f·ρ for the element of interest, where µ is the difference between the mass absorption coefficient above and below the absorption edge (g⁻¹ cm²), RE is the resonance enhancement factor (dimensionless), f is the mass fraction of the element within the solid phase, and ρ (g⁻¹ cm²) is the solid phase density (Table 2). Based on this approach, thicknesses of nearly all SOC in these maps are less than 100 nm. The regions with thicker C appear to be distinct SOC particles rather than coatings on mineral surfaces (Figure 1). Oxides of the major mineral elements Al and Si each exceeded 300 nm in thickness in the Cambisol sample, with their thicker regions interfering with mapping of C and other elements.
STXM maps from the Phaeozem reflect moderate correlations of C with Al, Si, and Fe (Figure 2a-c). The C-Fe correlation plot (Figure 2c) may actually reflect several different C associations; with Fe (hydr)oxides, with Fe-substituted aluminosilicates, and locations that have both C and Fe on a common substrate (e.g., an aluminosilicate). Figure 2f shows that for this sample, even Ca is better correlated to C than the aforementioned elements. Based on typical values of their cation exchange capacities, Ca\(^{2+}\)-saturated humic acids and smectites both contain about 2 mass% Ca. Although Ca\(^{2+}\) undoubtedly occupies a significant fraction of the cation exchange sites in SOC, the Ca/C ratios are about an order of magnitude greater than that attributable to cation exchange. Spectroscopic evidence ruling out CaCO\(_3\) as an explanation for C-Ca correlations in this sample is discussed later. In the case of the Cambisol maps, some associations between C and mineral cations are obscured by the presence of very thick aluminosilicate particles, such that the C correlation plots (Figure 3) and correlation coefficients (Table 3b) are less informative. All areas within the Ultisol maps were thin enough to examine C associations with mineral cations, and correlations with Al, Si, and Fe were nearly equally strong (Figure 4a-c, and Table 3c). A number of other element-element data pairs had only very weak correlations (Tables 3a-c).

Upon inspecting the various elemental correlations (Figures 2-4 and Tables 3a-c), it is clear that those between Al and Si OD values are the strongest. This resulted from the prevalence of aluminosilicates in the clay-size fractions of these soils, and because Al and Si are mapped at the highest (most penetrating) X-ray energies. Molar ratios of Si:Al were calculated by factoring their atomic weights into their RE\(\mu\) ratios, and plotted on the Si-Al correlation graphs (Figures 2d, 3d, and 4d). In the Phaeozem, the STXM data are in good agreement with 2:1 ratios, expected based on the predominance of smectite in its clay fraction (Table 1). The
Cambisol Al and Si data correlated very well with the 1:1 line, within the range possible for kaolinite and vermiculite (Dixon and Weed, 1989), two dominant clay minerals in the bulk soil (Table 1). The Si:Al ratios obtained for the Ultisol specimen are in good agreement with 1.7:1, typical of illite (Dixon and Weed, 1989). The possibility that illite, the main mineral phase in the bulk Ultisol (Table 1), is indicated by the measured Si:Al ratios measured in this sample is further supported by the good correlations between K, Al, and Si, with ratios typical for illite. The measured K-Si ratios (Figure 4f) cluster around the range of 5.4 expected for illite (Dixon and Weed, 1989).

The OD values for Fe are well correlated to both Al and Si in the maps obtained on suitably thin samples. In the Phaeozem (Table 3a) and Ultisol (Table 3c), Fe correlation coefficients with Al and Si all exceeded 0.82. The Al:Fe ratios were approximately 2:1 in the Phaeozem (Figure 2e), and 3:1 in the Ultisol (Figure 4e). These fairly linear relations and moderately high Fe fractions may be primarily attributable to Fe substitutions for Al in gibbsite sheets within 2:1 clays. For comparison, Al:Fe ratios of about 3:1 for montmorillonite, and about 3.2 to 5.1 for illite have been reported (Dixon and Weed, 1989). Fe-(hydr)oxide coatings on aluminosilicates may also contribute to this correlation. Because of excessive thickness, Fe relations with Al and Si could not be examined for the Cambisol sample (e.g., Figure 3e).

**Carbon speciation**

Data presented up to this point have addressed spatial distributions of C and its pixel-scale associations with other elements, but not C speciation. Spatially resolved C speciation can reveal organic versus inorganic C, forms of SOC associated with soil minerals, and the extent of humification. Carbon NEXAFS spectra from specific locations within maps and for whole
specimens are presented in Figures 5-7. The dashed vertical lines within vertical shade bars in the plots of spectra indicate midrange values and ranges of energies, respectively, for peaks associated with specific C functional groups (Table 4). Also appearing in the higher energy range of these spectra are peaks from L_{III} and L_{II} edges of potassium. Absorption intensities are presented directly as OD values, but offset vertically to facilitate comparisons.

The distribution of SOC in the Phaeozem is both diffuse and locally concentrated in chemically distinct particulate forms (Figure 5). Although the two most concentrated C regions are attached to the mineral particles forming the microaggregate framework, they do not appear to coat Al-, Si-, or Fe-containing grains (Figure 1). Both of the C-rich regions are 400 to 600 nm in lateral extent (Figure 1), and about 100 nm thick (Figure 2). Region 1 has distinct peaks at 285.1 eV (aromatic C) and 288.0 eV (0.2 eV below the peptide peak energy), and a shoulder at 286.7 (phenolic, ketonic C). In contrast, the absorption edge for region 2 begins to rise about 1 eV below the aromatic C energy in the vicinity of a peak reported for quinonic C, exhibits a broad peak at 285.8 eV, and has additional peaks at 288.5 (carboxylic C) and 290.3 eV (carbonate/carbonyl C). Quinonic C occurs during decomposition of lignin (STEVenson, 1994), and thus may be more prevalent in larger SOC particles undergoing humification. The nature of the broad peak at 285.8 eV and the high Ca concentration within region 2 are unknown. CaCO_{3} is unlikely to be present because of the slightly acidic soil pH, and because of the weakness of the resonance at 290.3 eV relative to that of calcite (BENZERARA et al., 2005). The ubiquitous occurrence of this resonance at 290.3 eV and lack of C-Ca correlation suggest that this weak peak is indicative of some other carbonate or carbonyl groups in SOC. Other regions on this Phaeozem sample exhibited spectra that were similar to that of the whole specimen, with weak to moderate peaks at 285.1, 286.7, 288.5, and 290.3 eV.
Carbon NEXAFS were obtained over most of the Cambisol sample, excluding the regions with thickest Al and Si (particles “A” and “B” in the Figure 6 images). Like the Phaeozem sample, C is distributed very heterogeneously, with locally concentrated regions each having different NEXAFS spectra. Region 1 is the only location on this sample having a peak at 285.1 eV (aromatic C), followed by a gradual rise in the absorption edge, and appears to be a distinct attached particle (≈ 200 to 500 nm) rather than a coating on a mineral grain (Figure 1). Both regions 2 and 3 occur as SOC coatings on a mineral grain, and have C NEXAFS with peaks at 286.7 eV (phenolic C) and at 288.2-288.5 eV (peptidic, carboxylic C). All other regions (regions 4 and 5 are shown in Figure 6, but 3 other coating regions were also checked) of this sample only had peaks in the 288.2-288.5 eV range, and were similar to the spectrum of the whole sample.

Carbon NEXAFS spectra from the Ultisol sample were generally weaker in intensity because of lower C concentrations. In this sample, C was relatively uniformly distributed, with no large (>100 nm) particles detected (Figure 1). The C NEXAFS spectra were similar throughout the sample, containing peaks at 285.1, 286.7, and 288.5 eV (Figure 7). Although total C concentrations are low in this sample, the resonance from aromatic C (285.1 eV) was strong. More intense peaks at 297 and 300 eV were obtained because of the higher K contents in this illitic soil.

Collectively, the C NEXAFS maps display both variability and homogeneity within individual microaggregates. The variability was largely associated with localized SOC-rich regions within microaggregates. These SOC-rich regions appear to be distinct particles several hundred nm in size, that have higher fractions of aromatic C than their surroundings. Outside of these locally C-rich locations, C NEXAFS spectra within individual microaggregates were
relatively similar. In the Phaeozem, the pervasive NEXAFS features were those of phenolic and
carboxylic C, while in the carboxylic peak was most common within the Cambisol sample. As
previously noted, the Ultisol sample displayed a diffuse distribution of aromatic, phenolic, and
carboxylic C peaks over all surfaces.

**Implications**

The scientific basis for predicting changes in soil OC storage is under active
development, and is a critical component for carbon management and for predicting climate
change. Quantifying grain-scale SOC distribution may help test mechanistic models for
predicting larger scale SOC retention in soils. Using the wide energy range accessible by the
ALS beamline 11.0.2 STXM, this study provided direct determinations of SOC microscale
distribution, speciation and spatial associations with major soil mineral elements Si, Al, Ca, K,
Ti, and Fe. Diverse C functional groups occurred together, and SOC was distributed as discrete
particles and coatings on soil minerals. This study also demonstrated the viability of the
approach of sample preparation through selecting particles with suitable thicknesses, instead of
making thin sections from soil microaggregates. The selectivity for particle thicknesses ≤ 100
nm is not expected to affect conclusions concerning SOC associations with mineral surfaces
because soil surface area is largely associated with finer colloidal particles. This method is
especially attractive because of its simplicity and because it minimizes possible changes
introduced with physical-chemical processing. Like many other microscale analyses in soils and
heterogeneous media in general, large numbers of local measurements are required in order to
quantify microscopic distributions underlying macroscopic properties. Studies of more soil types,
with multiple samples analyzed using different approaches will help develop a clearer
understanding of specific SOC-mineral associations. Thus, the simplicity of approach presented here makes it conducive to obtaining large numbers of measurements, and is complementary to other more laborious preparation methods.

ACKNOWLEDGMENTS

We thank Markus Kleber, Margaret Torn, and Asmeret Berhe of LBNL for helpful discussions and for providing soil samples. We appreciate Yongman Kim’s help for soil total organic carbon analyses. We appreciate Glenn Waychunas for internal review of the manuscript. We thank the three anonymous reviewers and the Associate Editor Donald Sparks for their constructive review comments. Funding of this work was provided by the Geosciences Division of the Basic Energy Science (BES) program, under the Office of Science, U.S. Department of Energy. This work was conducted at beamlines 11.0.2 and 5.3.2 at the Advanced Light Source, Lawrence Berkeley National Laboratory, which is supported by the Office of Science, Office of Basic Energy Sciences, Division of Materials Sciences, and Division of Chemical Sciences, Geosciences, and Biosciences of the U. S. Department of Energy under contract DE-AC03-76SF00098.
LITERATURE CITED


Table 1. Soil samples and characteristics. Depths are relative to the mineral soil surface. Clay mineralogy: cl chlorite, gt goethite, il illite, ka kaolinite, pl plagioclase, sm smectite, ve vermiculite. The pH values are from 1:1 soil:water extracts. Fe-dith. and Fe-ox. Refer to dithionite and oxalate extractable Fe (SIREGAR et al., 2005).

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Table 2. X-ray energies, differences between mass attenuation coefficients above and below the absorption edge ($\mu$), and approximate resonance enhancements (RE) used for elemental mapping. *Values of $\mu$, RE, and density for Al, Si, and Fe are for generic oxides Al(OH)$_3$, SiO$_2$, and Fe$_2$O$_3$, respectively, and therefore used in this study only to estimate thicknesses. **In order to estimate SOC thickness, $\rho = 1.4$ g cm$^{-3}$ and $f = 0.58$ were used (STEVENSON, 1994). $f$ is the mass fraction of the element of interest within its solid phase.

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<td>703.0</td>
<td>710.0</td>
<td>15,800*</td>
<td>5*</td>
<td>4.3*</td>
<td>0.63</td>
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Table 3. Correlation coefficients from pairs of OD values from different elements within maps of (a.) Phaeozem, (b.) Cambisol, and (c.) Ultisol.

<table>
<thead>
<tr>
<th></th>
<th>Al</th>
<th>Si</th>
<th>K</th>
<th>Ca</th>
<th>Ti</th>
<th>Fe</th>
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<tbody>
<tr>
<td><strong>a. Phaeozem</strong></td>
<td></td>
<td></td>
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<tr>
<td>C</td>
<td>0.547</td>
<td>0.445</td>
<td>0.442</td>
<td>0.767</td>
<td>0.450</td>
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<tr>
<td>Al</td>
<td>0.876</td>
<td>0.344</td>
<td>0.740</td>
<td>0.319</td>
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<td>Si</td>
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<td>0.686</td>
<td>0.241</td>
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<tr>
<td>K</td>
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<td>0.412</td>
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<td>Ca</td>
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<td><strong>b. Cambisol</strong></td>
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<tr>
<td>C</td>
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<td>0.039</td>
<td>0.464</td>
<td>0.339</td>
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<td><strong>c. Ultisol</strong></td>
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<tr>
<td>C</td>
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<td>0.816</td>
<td>0.706</td>
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Table 4. Carbon functional groups, peak energy ranges, transitions, and literature sources.

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<th>C functional groups</th>
<th>Energy, eV</th>
<th>transition</th>
<th>sources</th>
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<tr>
<td>Quinonic C=O, protonated aromatic</td>
<td>283.7 – 284.3</td>
<td>1s-π*</td>
<td>(CODY et al., 1998; FRANCIS and HITCHCOCK, 1992; LEHMANN et al., 2005)</td>
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<tr>
<td>Aromatic ( \text{C}<em>{\text{arom}}=\text{C}</em>{\text{arom}} ), ( \text{C}_{\text{arom}}=\text{H} )</td>
<td>284.9 – 285.5</td>
<td>1s-π*</td>
<td>(BENZERARA et al., 2004; CODY et al., 1998; LEHMANN et al., 2005; SCHUMACHER et al., 2005)</td>
</tr>
<tr>
<td>Phenolic ( \text{C}_{\text{arom}}-\text{OH} ), ketonic C=O</td>
<td>286.5 – 287.2</td>
<td>1s-π*</td>
<td>(BENZERARA et al., 2004; BOYCE et al., 2002; LEHMANN et al., 2005; SCHUMACHER et al., 2005)</td>
</tr>
<tr>
<td>Aliphatic C-H</td>
<td>287.1 – 287.8</td>
<td>1s-3p/σ*</td>
<td>(BOYCE et al., 2002; LEHMANN et al., 2005)</td>
</tr>
<tr>
<td>Amide-carbonyl (peptidic) C=O</td>
<td>288.2</td>
<td>1s-π*</td>
<td>(BENZERARA et al., 2004)</td>
</tr>
<tr>
<td>Carboxylic C=O, C-OH</td>
<td>287.7 – 288.6</td>
<td>1s-π*</td>
<td>(BENZERARA et al., 2004; BOYCE et al., 2002; LEHMANN et al., 2005; SCHUMACHER et al., 2005)</td>
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<tr>
<td>Carbonate, carbonyl C=O</td>
<td>290.2 – 290.5</td>
<td>1s-π*</td>
<td>(BENZERARA et al., 2005; BENZERARA et al., 2004; SCHUMACHER et al., 2005)</td>
</tr>
</tbody>
</table>
FIGURE CAPTIONS

Figure 1. Elemental relative distribution maps within microaggregates from the Phaeozem, Cambisol, and Ultisol (rainbow color scale, with red = highest concentration). The overall microaggregate outline for each sample is shown in the optical density map collected at 710 eV.

Figure 2. Phaeozem sample correlation plots of OD obtained from STXM maps shown in Figure 1. Linear correlation coefficients for these plots and other elemental relations are provided in Table 3a.

Figure 3. Cambisol sample correlation plots of OD obtained from STXM maps shown in Figure 1. Linear correlation coefficients for these plots and other elemental relations are provided in Table 3b.

Figure 4. Ultisol sample correlation plots of OD obtained from STXM maps shown in Figure 1. Linear correlation coefficients for these plots and other elemental relations are provided in Table 3c.

Figure 5. Carbon NEXAFS spectra obtained within selected areas of the Phaeozem microaggregate (regions numbered 1 through 6), and for the whole sample. Spectral features identified by the vertical dashed lines correspond to C in (a) quinonic, (b) aromatic, (c) phenolic, (d) aliphatic, (e) peptidic, (f) carboxylic, and (g) carbonate/carbonyl functional groups. The shaded gray bands indicate energy ranges attributed to each functional group (Table 4). The
peaks at the higher energies result from small amounts of $K^+$, and correspond to its $L_3$ and $L_2$
edges.

Figure 6. Carbon NEXAFS spectra obtained within selected areas of the Cambisol microaggregate (1 through 5), and for the whole sample. The aluminosilicate particles that are too thick for C NEXAFS measurements are labeled A and B in the maps. Spectral features identified by the vertical dashed lines correspond to C in (a) quinonic, (b) aromatic, (c) phenolic, (d) aliphatic, (e) peptidic, (f) carboxylic, and (g) carbonate/carbonyl functional groups. The peaks at the higher energies are from $K^+$, and correspond to its $L_3$ and $L_2$ edges.

Figure 7. Carbon NEXAFS spectra obtained within a selected area of the Ultisol microaggregate (region 1), and for the whole sample. Spectral features identified by the vertical dashed lines correspond to C in (a) quinonic, (b) aromatic, (c) phenolic, (d) aliphatic, (e) peptidic, (f) carboxylic, and (g) carbonate/carbonyl functional groups. Peaks from $K^+$ $L_3$ and $L_2$ edges are more intense in this sample because of the K-rich illite matrix.
Figure 1. Elemental relative distribution maps within microaggregates from the Phaeozem, Cambisol, and Ultisol (rainbow color scale, with red = highest concentration). The overall microaggregate outline for each sample is shown in the optical density map collected at 710 eV.
Figure 2. Phaeozem sample correlation plots of OD obtained from STXM maps shown in Figure 1. Linear correlation coefficients for these plots and other elemental relations are provided in Table 3a.
Figure 3. Cambisol sample correlation plots of OD obtained from STXM maps shown in Figure 1. Linear correlation coefficients for these plots and other elemental relations are provided in Table 3b.
Figure 4. Ultisol sample correlation plots of OD obtained from STXM maps shown in Figure 1. Linear correlation coefficients for these plots and other elemental relations are provided in Table 3c.
Figure 5. Carbon NEXAFS spectra obtained within selected areas of the Phaeozem microaggregate (regions numbered 1 through 6), and for the whole sample. Spectral features identified by the vertical dashed lines correspond to C in (a) quinonic, (b) aromatic, (c) phenolic, (d) aliphatic, (e) peptidic, (f) carboxylic, and (g) carbonate/carbonyl functional groups. The shaded gray bands indicate energy ranges attributed to each functional group (Table 4). The peaks at the higher energies result from small amounts of K⁺, and correspond to its L₃ and L₂ edges.
Figure 6. Carbon NEXAFS spectra obtained within selected areas of the Cambisol microaggregate (1 through 5), and for the whole sample. The aluminosilicate particles that are too thick for C NEXAFS measurements are labeled A and B in the maps. Spectral features identified by the vertical dashed lines correspond to C in (a) quinonic, (b) aromatic, (c) phenolic, (d) aliphatic, (e) peptidic, (f) carboxylic, and (g) carbonate/carbonyl functional groups. The peaks at the higher energies are from K⁺, and correspond to its L₂ and L₃ edges.
Figure 7. Carbon NEXAFS spectra obtained within a selected area of the Ultisol microaggregate (region 1), and for the whole sample. Spectral features identified by the vertical dashed lines correspond to C in (a) quinonic, (b) aromatic, (c) phenolic, (d) aliphatic, (e) peptidic, (f) carboxylic, and (g) carbonate/carbonyl functional groups. Peaks from $K^+$ L$_3$ and L$_2$ edges are more intense in this sample because of the K-rich illite matrix.