Identification of organic matter in soil aggregates using SR-$\mu$CT

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Knowledge of the location of soil organic matter (SOM) and its spatial association to soil structure is an important step in improving modeling approaches for simulating organic matter turnover processes. Advanced models for carbon mineralization are able to account for the 3D distribution of SOM (Monga et al. 2008), however, their application is still limited by the fact that no method exists to determine the spatial distribution of SOM in undisturbed soils. Recent research suggests that physical protection is a major factor in controlling mineralization rates (Six et al. 2002) and subsequently soil organic matter sequestration. Non-invasive 3D visualization of SOM distribution could significantly enhance the assessment of the role of physical protection of organic matter in carbon cycling. However, traditional micromorphology analysis such as soil thin sectioning bear the risk of disturbing the soil structure thus changing mineral – organic matter associations and are inherently 2-dimensional lacking information on the topology of the pore space through which oxygen, water and dissolved organic matter (DOM) can diffuse to microbially active sites in the pore space.

SR-based X-ray microtomography is an advanced and promising tool in gaining knowledge on the 3-dimensional organization of soil constituents which on a voxel level could be implemented into spatially explicit models. However, since the contrast of linear attenuation coefficients of soil organic matter on the one hand and mineral components and water on the other hand are relatively low, especially when soil constituents are finely dispersed, organic matter within the soil pore space is often not resolved in ordinary X-ray absorption contrast imaging. This shortcoming could potentially be circumvented by scanning samples at carbon edges, however, occurring at low energies (< 1keV) the beam intensity is not sufficient to fully penetrate the sample. Therefore we have developed a staining procedure for organic matter using an element with an absorption edge at a higher energy level.

Osmium is known from transmission electron microscopy analysis (TEM) to stain organic matter specifically and irreversibly while having an absorption edge at approximately 74 keV.

First results of this project builds upon a previous study where the influence of small scale pore space architectures on the decomposition/mineralisation of organic matter in intra-aggregate pore volumes was investigated (Ngom et al. 2011). Here we report on the application of a novel Osmium vapor staining method to analyze differences in organic matter content and identify small scale spatial distribution of SOM in soil aggregates. We have taken soil aggregate samples (6-8 mm across) obtained from an arable subsoil (40-50 cm depth, Haplic Luvisol derived from loess) under Alfalfa crop vegetation. Aggregate samples were investigated by synchrotron-based X-ray microtomography (SR-$\mu$CT) after staining the sample with Osmium (OsO₄) vapor. For staining with osmium vapor aggregates were placed above a 2% w/w osmium solution for approximately 52h at ambient temperature (~20 °C) in a closed vial under a fume hood. We utilized the monochromatic X-ray beam to locate osmium bound to SOM in the stained soil aggregate samples by scanning the aggregates at different photon energies: 30 keV at which the attenuation contrast is optimal for distinguishing other soil constituents, 70 keV (below the absorption of osmium) and 78 keV (above the absorption edge of osmium). Image sections which indicated changes in contrast at the absorption edge of osmium are exemplary shown in Fig. 1. The results suggest that particulate organic matter, which was not visibly by scanning samples at energy levels optimal for resolving soil structure (30 keV), was identified after osmium staining and scanning the samples at energy levels just below (70 keV) and above (78 keV) the absorption edge of Osmium. Subtracting the images below from the images above the osmium edged we were able to isolate stained particulate organic matter which otherwise would have been wrongly interpreted as pore space without staining (Fig. 2). Data processing is still under progress, but the first results shown here prove the potential to localize organic matter non-invasively in 3D.
Fig. 1: Reconstructed image sections of two different stained soil aggregates (A1, A2) scanned at different photon energies (30, 70 and 78 keV). Sections indicating changes in contrast of the image at the absorption edge of osmium are noticed e.g. within the blue boxes.

Fig. 2: 3D image plots representing osmium stained particulate organic matter identified by the contrast of the images at the osmium absorption edge. Osmium stained organic matter was visualized in 3D by subtracting selected 3D image sequences of 70 keV (below the absorption edge) from corresponding 78 keV 3D image sequences (above the absorption edge) according to Fig. 1 (blue boxes).

**Literature:**