Non-invasive localization of soil organic matter by Osmium staining using SR-μCT

S. Peth¹, C. Chenu², P. Garnier³, N. Nunan², V. Pot³, F. Beckmann⁴, M. Ogurreck⁴, R. Horn¹

¹ Institute of Plant Nutrition and Soil Science, University of Kiel, Olshausenstr. 40, D-24118 Kiel, Germany
² INRA, Centre de Grignon, Biogéochimie et écologie des milieux continentaux, 78850 Thiverval Grignon, France
³ INRA, Centre de Grignon, UMR environment et grandes cultures, BP 01, 78850 Thiverval Grignon, France
⁴ GKSS-Research Centre, Max-Planck-Str. 1, 21502 Geesthacht, Germany

Carbon sequestration in soil repositories has become a major topic in climate change debates. It is suggested that soil physical infrastructure plays a key role in storing and remobilizing organic carbon within soils. Especially aggregation is known to have a positive effect on carbon stabilization [1]. Stabilization is most effective in small (millimetre scale) naturally formed soil aggregates. For instance, it was found that particulate organic matter (POM) is physically occluded in pores with diameters of 2-5 µm suggesting an interaction between the physical function of micro-aggregate infrastructures and long term carbon stabilization in mineral and organic matter associations [2]. Apart from a physical lockup of POM fractions, biogeochemical sorption of soluble organic carbon compounds is also strongly controlled by transport processes within intra-aggregate pore networks. Moreover, not only the diffusion of dissolved C but also nutrients, oxygen, water and heat to soil microbes residing in microsites determine the fate of soil carbon and its long-term sequestration in soils [3].

Current model predictions of carbon dynamics in soil are often contradictory. A major drawback is that the physical structure of soils is not implemented in most carbon models which usually work on a number of functional pools [4] widely ignoring actual pore space geometries. Recently developed geometry-based models allow the simulation of the spatial distributions of microorganisms and organic matter in the 3D soil pore space [5]. However, so far spatial distribution of organic matter within pore spaces as an input parameter is not based on measurements but achieved by some simplifying assumptions. The reason for this is that soil organic matter (SOM) is difficult to be observed in situ. Although very fine scale spatial distribution of organic matter has recently been explored using TEM-EDS methods [6] a gap in this method is that the spatial distribution of organic carbon in 3D is still not known and that only an incomplete section of the soil aggregate can be investigated neglecting the influence of all not sampled pore regions. X-ray tomography would be a potential tool but on the other hand is limited for the detection of the normally low concentrations of SOM in mineral soils (1-5 wt.-%) due to the low attenuation contrasts of SOM compared to the other compounds and its close association with clay mineral surfaces.

To overcome this limitation we developed an approach to locate SOM in natural soil aggregates in situ with SR-μCT using a staining method applied in electron microscopy [6]. We selected osmium as a staining agent which reacts with unsaturated C-bonds of organic compounds including finely disseminated organic matter often absorbed onto clay mineral surfaces and not visible as discrete particles (POM) [6]. The benefit of using osmium is that it is an element with a high atomic number (Z=76) having an absorption edge at a photon energy of ~74 keV. By making advantage of the monochromatized X-ray beam we were able to locate SOM non-invasively in the stained samples with X-ray microtomography (SR-μCT) by scanning at various photon energies: 1) above the absorption edge at 78 keV, 2) below the absorption edge at 70 keV, and 3) at 30 keV where attenuation contrast is optimal for distinguishing other soil constituents in order to resolve soil structure. Differences in X-ray attenuation between the scans above and below the absorption edge are related to osmium bound to organic matter in the sample. In this study we used two air dry soil aggregate samples with an estimated (based on other samples from the same treatment) content of SOM of >2 wt.-% (A1) and <1 wt.-% (A0), respectively. The aggregates were exposed to an OsO₄ solution for 48h at ambient temperature (~20 °C) in a closed vial under a hood. Osmium vapor passed through the pore space of the soil aggregates where osmium was fixed irreversible to organic matter compounds. SR-μCT scanning was done at beamline W2 (HARWI II) at a voxel resolution of 9.77 µm.
The different contents of SOM of the two soil aggregates were clearly visible by a higher fraction of bright areas in the sample with more organic matter (A1) due to a higher X-ray attenuation of the osmium stain (Fig. 1). Note that although the stain appeared to be distributed over the whole sample (Fig. 1a) some other mineral parts remained completely unstained (darker grey scale values) indicating a heterogeneous distribution of the stain. However, not all bright grey scale values are necessarily be attributed to stained organic matter since other soil minerals may also be associated with high density elements (e.g. Fe-containing minerals) thus likewise resulting in a higher attenuation of the X-ray beam. An objective localization of SOM can only be obtained by comparing the images scanned at photon energies below (70 keV) and above (78 keV) the absorption edge of osmium which indicates the distribution of osmium in the sample which only binds to organic carbon (Fig. 1).

![Figure 1: Reconstructed images of two osmium stained soil aggregates with a) more and b) less organic matter.](image)

Figure 1: Reconstructed images of two osmium stained soil aggregates with a) more and b) less organic matter. The left part shows optimal attenuation contrast where both the bright osmium stained areas, other mineral constituents and the pore space is very well resolved. The middle and the right part indicate the change in contrast of the image at the absorption edge of osmium reflecting the spatial distribution of stained organic matter in the sample.

References