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Short communication

Localization of soil organic matter in soil aggregates using synchrotron-based X-ray microtomography

Stephan Peth [a,*], Claire Chenu [b], Nelly Leblond [b], Anneka Mordhorst [c], Patricia Garnier [d], Naoise Nunan [b], Valérie Pot [d], Malte Ogurreck [e], Felix Beckmann [e]

[a] *Department of Soil Science, University of Kassel, Nordbahnhofstr. 1a, D-37213 Kassel, Germany*
[b] *AgroParisTech, CNRS, UMR7618 Biogéochimie et écologie des milieux continentaux, Campus AgroParisTech, 78850 Thiverval Grignon, France*
[c] *Institute for Plant Nutrition and Soil Science, Christian-Albrechts-University Kiel, Hermann-Rodewaldstr. 2, 24118 Kiel, Germany*
[d] *INRA, UMR environment et grandes cultures, 78850 Thiverval Grignon, France*
[e] *GKSS-Research Centre, Max-Planck-Str. 1, 21502 Geesthacht, Germany*

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ABSTRACT

Modelling carbon mineralisation in natural soils is a major topic in soil and climate research. Current models need to be improved to include soil structure as an influencing factor to better predict C fluxes between pedosphere and atmosphere and to estimate carbon sequestration potentials. Geometry-based mechanistic modelling approaches have recently been developed to systematically study the effect of soil structure on carbon decomposition. Such models require spatially explicit input parameters describing the architecture of the pore space and the heterogeneous distribution of microbes and organic matter as decomposable substrate. The latter is very difficult to determine *in situ*, resulting in increased uncertainty in the models. To obtain more realistic input data, we have developed a novel approach to locate soil organic matter (SOM) in undisturbed aggregates of soil using a combination of synchrotron-based X-ray microtomography and osmium as a staining agent for SOM. Here, we present the first results using 5 mm sized soil aggregate samples with contrasting C-contents in which we obtained maps of organic matter distributions in relation to the pore networks at the aggregate scale.

Soil respiration represents the second largest C flux to the atmosphere with nearly 10% of the atmosphere's CO_2 cycling through soil (e.g. Schimel et al., 1996). Estimations of the carbon sink potential remain difficult and the prediction of long term soil C dynamics has recently been questioned because the stabilization mechanisms of soil organic matter (SOM) and its relation to soil structure is still an overlooked factor in most SOM modelling approaches (Six et al., 2002). This is surprising since aggregation is well known to have a positive effect on carbon stabilization (Lal, 2004) where small (sub-millimetre scale) naturally formed soil aggregates seem to be the most effective storage sites (Six et al., 2002). Chenu and Plante (2006), using transmission electron microscopy, and Kinyangi et al. (2006), using synchrotron-based scanning transmission X-ray microscopy, found that small particles of organic matter are physically occluded in micropores, underlining the importance of microscale structures. The physical properties of micro-aggregate structures are intimately intertwined with long term carbon stabilization thanks to organo-mineral associations. Smucker et al. (2010) also argued that soil physical structure plays a key role in storing and remobilizing organic carbon within soils. Functionally, the pore space architecture controls the fate of soil C by (i) determining the accessibility of substrate to microbial cells or enzymes through forming physical barriers (Killham et al., 1993) and (ii) influencing microbial activity through the diffusion of oxygen into aggregates, mainly related to blocking of pores with water menisci (Sexstone et al., 1985). Besides the physical lockup of OM fractions, sorption of soluble organic carbon compounds also depends on transport processes within intra-aggregate pore networks. However, the long-term sequestration of carbon in soils is determined not only by the diffusion of dissolved C but also by the diffusion of nutrients, oxygen, water and heat to soil microbes residing in microsites (Chenu and Stotzky, 2002; Smucker et al., 2007).

Usually carbon models are based on a number of kinetic pools and ignore the physical structure and pore space geometry of soils. Recently, more advanced models have been developed, based on

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[*] Corresponding author. Tel./fax: +49 5542 98 1590.
E-mail address: peth@uni-kassel.de (S. Peth).

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the hypothesis that carbon mineralisation is a function of its accessibility and location within soil pore spaces (Kuka et al., 2007; Monga et al., 2008). A first attempt at simulating carbon decomposition using a 3D geometry-based model was proposed by Monga et al. (2008). It is a spatially explicit model in which the spatial distributions of microorganisms and organic matter are simulated in 3D soil pore space. However, to date the spatial distribution of organic matter within pore space, an important input parameter in these models, has not been based on measurements but rather on some simple assumptions (Ngom et al., 2011). The reason for this is that soil organic matter (SOM) is difficult to observe *in situ*. Although very fine scale spatial distribution of organic matter has been measured recently using TEM-EDS methods (Chenu and Plante, 2006), only an incomplete representation of SOM distribution in soil aggregates is obtained as the 3D nature of soils is not accounted for. X-ray microtomography (XCMT) is a potential tool to non-invasively measure 3D maps of X-ray attenuation which is related to local material densities (Peth, 2010). Theoretically, this could be used to infer the spatial distribution of solid soil constituents (mineral and organic) as well as water and air-filled pores. It has been shown that the method is suitable for resolving intra-aggregate pore space architectures down to a scale relevant for microbial processes (Feeney et al., 2006; Nunan et al., 2006; Peth et al., 2008a). However, the X-ray attenuation coefficients of organic matter fall between those of pores (water or air filled) and the mineral matrix, making phase separation (segmentation) very difficult (De Gryze et al., 2006; Kaestner et al., 2006). Therefore, *in situ* detection of typically low concentrations of SOM in arable mineral soils (1–5 wt.-%) is limited due to the weak X-ray attenuation contrasts of SOM compared to the other compounds and its close association with clay mineral surfaces.

To overcome this limitation we propose a new approach to locate SOM in natural soil aggregates *in situ,* using synchrotron-based X-ray microtomography (SR-μCT) in combination with a staining method previously applied in electron microscopy of soils (Chenu and Plante, 2006). We selected osmium as a staining agent as this reacts with unsaturated C-bonds of organic compounds (Hayes et al., 1963), such as those present in lipids, lignins and proteins (Behrman, 1987), including finely disseminated organic matter often absorbed onto clay mineral surfaces and not visible as discrete organic particles (Chenu and Plante, 2006). The benefit of using osmium is that it has a high atomic number ($Z = 76$) and a distinct absorption edge at a photon energy of ~74 keV, making it easily distinguishable from other material in soil. Making use of the monochromatic X-ray beam available at synchrotrons, we were able to locate SOM in the stained soil aggregate samples in relation to soil structure by scanning at photon energies above the absorption edge (78 keV), below the absorption edged (70 keV) and at 30 keV, where attenuation contrast is optimal for distinguishing other soil constituents (Fig. 1), in order to resolve soil structure (Peth et al., 2008b). Differences in X-ray attenuation at energies above and below the absorption edge are predominantly related to osmium bound to organic matter in the sample. Thus, by measuring osmium in the samples we will be able to infer the 3D distribution of organic matter in the intra-aggregate pore space. 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.

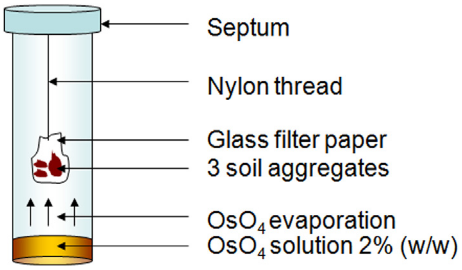

Fig. 2. Schematic showing the Osmium vapour staining procedure of soil aggregates.

Several soil aggregates were vapour stained by exposing them to a 2% w/w OsO_4 solution for 48 h at ambient temperature (~20 °C) in a closed vial under a fume hood (Fig. 2). Osmium vapour passed through the pore space of the soil aggregates where osmium was fixed irreversibly to organic compounds. Based on quantitative estimates of Os fixation on biological samples (Hayes et al., 1963; Sousa et al., 2008), we calculated that the amount of Osmium present in the vial was largely in excess of the amount required to react with all the aggregates' organic matter. To verify the vapour staining of soil organic matter and determine the optimum staining time, we observed stained aggregates using a Philips 525 SM Scanning

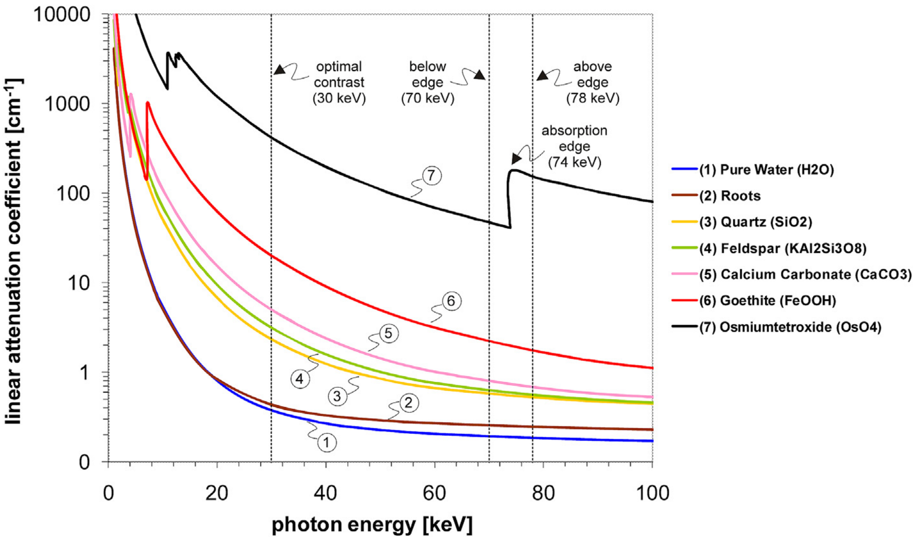

Fig. 1. Calculated linear attenuation coefficients for major soil minerals, organic matter (roots) water and osmium. Vertical dashed lines indicate the scanning energies. Attenuation coefficients were calculated using the Photon Cross-Section Database (XCOM).