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## Visualization of soil particulate organic matter by means of X-ray CT?

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The role of soil structure in organic matter (OM) stabilization has been primarily investigated through physical fractionation studies operative at the scale of aggregates and smaller organo-mineral particles. By narrowing down soil structure to an arrangement of mineral and organic particles, the majority of studies did not explore the spatial organization of the soil pore network, the actual habitat of microorganisms. The pore structure of soil can have a significant impact on soil processes like OM decomposition by excluding OM from micro-organisms in small pores, by regulating the diffusion of substrates and metabolites and by regulating aeration and presence of moisture.

Because of its ability to visualize the 3D architecture of soil non-destructively, X-ray Computed Tomography (CT) is becoming a widespread tool for studying soil pore network structure. However, phase determination of pore space, soil OM, soil mineral matter (MM) and water is often limited even with the latest technological and software advances, allowing high resolution and better quality imaging. Contrast agents commonly used in histology enable enhancement of X-ray attenuation of targeted structures or compounds. Here we report on the first systematic investigation of the use of such X-ray contrast agents for soil research. An evaluation procedure as well as a method to apply the agents to soil samples was developed and applied on reference soil samples. The effectiveness and selectivity of the contrast agents was evaluated for soil organic matter (SOM), MM and water. Several products were found to selectively increase the attenuation of water or SOM. The four agents with the best OM-staining capabilities (Phosphomolybdenic acid (PMA), silver nitrate, lead nitrate and lead acetate) were further tested on an OM-MM mixture. Observed differences in reactivity of the staining agents with MM components were apparent, suggesting that contrasting agents may have to be selected for the specific composition of the soil mineral matrix. Furthermore, techniques such as multiple-energy scanning and K-edge imaging, even in the future perhaps in combination with spectral resolving detectors or spectroscopic techniques can could further enhance the potential benefit from this study of X-ray CT staining agents. The high Z elements of the staining agents have unique and characteristic traits that can be detected or quantified with the abovementioned techniques and methods. We conclude that, given resolution limits and inherent presence of partial volume effects staining, X-ray CT-based localization of discrete SOM particles will be limited to a lower limit of 20-50 μm. Still, the improved 3D visualization of OM and soil pore space opens up possibilities for tailored lab experiments with measures of microbial activity, which could generate new insights in carbon cycling at small scales.

In addition, we report on a lab incubation experiment in which  $CO_2$  respiration from soil cores was monitored (headspace GC analysis) and an X-ray CT approach yielded soil pore size distributions. We incubated a sandy loam soil (with application of ground grass or sawdust) in 18 small aluminium rings (Ø 1 cm, h 1 cm). Bulk density was adjusted to 1.1 or 1.3 Mg m-3 (compaction) and 6 rings were filled at a coarser Coarse Sand:Fine Sand:Silt+Clay ratio. While compaction induced a strong reduction in the cumulative C mineralization for both grass and sawdust substrates, artificial change to a coarser soil texture only reduced net C mineralization from the added sawdust. There thus appears to be a strong interaction effect between soil pore structure and substrate type on substrate decomposition. Correlation coefficients between the C mineralization rates and volumes of 7 pore size classes (from the X-ray CT data) also showed an increasing positive correlation with increasing pore size. Since any particulate organic matter initially present in the soil was removed prior to the experiment (sieving, ashing the >53 $\mu$ m fraction and recombining with the <53 $\mu$ m fraction), the added OM can be localized by means of X-ray CT. Through on-going image analysis the surrounding porosity of the added grass or sawdust particles is being quantified to further study the interaction between the soil pore structure and substrate decomposition.