X-ray fluorescence tomographic imaging of bacteria within soil aggregates

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Microbial metabolism in soil is a primary driver of the terrestrial carbon cycle, controlling nutrient availability to plants, contaminant remediation, water quality, and other ecosystem processes. However, our understanding of metabolic processes such as nutrient uptake rates, substrate preferences, and the distribution of microbes and microbial metabolism in soil are limited by the opacity and complex three-dimensional structure of soil. We have developed a novel approach using x-ray fluorescence tomography to image in three dimensions the distribution of bacteria labeled with quantum dots within soil aggregates. Specifically, we have conjugated a variety of amino acids to CdSe/ZnS (core/shell) quantum dots that are less than 5 nm in size and surface functionalized with carboxyl or hydroxyl groups. We have used a suite of imaging techniques to identify uptake of these quantum dots by bacteria, and the location of bacteria in soil. First, we used aberration-corrected TEM imaging to verify preferential uptake of the conjugated quantum dots over unconjugated dots by bacteria. Confocal microscopy was used to quantify average quantum dot uptake by bacteria for different amino acids conjugated to the quantum dots. Next, we used two dimensional x-ray fluorescence microscopy to identify colocalization of macronutrients (P, S, Cl, Ca) and quantum dots (Se), demonstrating that bacteria concentrate sufficient quantum dots to facilitate detection using the x-ray fluorescence technique. Finally, after allowing 5 days for the quantum-dot labeled bacteria to colonize submillimeter-sized soil aggregates, we used x-ray fluorescence tomography to identify the distribution of bacteria within the pore structure of the soil aggregate by monitoring the Se Kα tomographic signal from the aggregate. This work, for the first time, demonstrates an ability to image the distribution of micron-sized soft tissue and, potentially, metabolic processes, within opaque and electron dense media without use of thin sectioning approaches. These results, additional experimental protocols, and data analysis approaches will be presented.