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Developing a Practical Framework for Spatially Correlated Electron and X-ray High Resolution Chemical Imaging

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Soil microbial communities affect the formation of micro-scale organo-mineral associations where complex processes, such as soil organic matter stabilization occur in a narrow zone of biogeochemical gradients. We designed a field study to examine these processes using in-growth mesh bags containing minerals of selected classes, with the goal to analyze the direct interactions between microbes and minerals, and to identify the nature of the newly formed organo-mineral associations [1]. Early results support the in-situ formation of organic compounds by microbes in the mesh bags, and indicate less stable organic carbon pools than found in the bulk soil.

To learn about the nature of organo-mineral associations on soil mineral surfaces, we developed a method for spatially correlated electron and X-ray chemical imaging. This method can be applied to a variety of materials from both life and physical science, or – like in this case, their mix.

Spatially-resolved chemical and x-ray imaging on different instruments was employed, in addition to the bulk mass spectroscopy methods such as Fourier transform Fourier transform ion cyclotron resonance mass spectrometry (FTICR – MS) used for determining the molecular composition of microbial-mineral associations, by extracting organic material by a series of different polarity solvents [2]. Tecnai T-12 TEM was selected as a relatively high-throughput imaging tool for surveying large grid areas, to obtain maps of regions of interest (ROI). However, to provide a statistically sound characterization of the microbial-mineral interactions, we needed to identify, image and map series of regions of interest for follow-up analyses. The microbes are not uniformly distributed on a TEM grid, and the current workflow consists of systematically mapping a grid, saving the positions, and then revisiting these areas with a suite of the respective imaging and analytical measurements [3].

Low dose imaging scheme was used, to minimize the radiation damage that would unquestionably occur during the material exposure to the electron and ion beam. The radiation damage was the main concern in regards of the material integrity, and it dictated the order of the three successive imaging and spectroscopical methods. After obtaining the ROI map, the TEM grid was mounted on an aluminum STXM plate and transferred to the Advanced Light Source laboratory (Berkeley, CA). The grid was imaged by STXM instrument (beamline 5.3.2.2), first locating the ROIs, then running the X-ray absorption near edge structure spectroscopy (XANES) on the respective energy edge of interest (C, Ca and Fe). Rotation, translation and sometimes an image flip needed to be done to project the same image orientation as the original mapped image. The radiation damage caused by soft X-ray spectroscopy has been previously discussed, but we didn't observe any visible material changes. The grid was then carefully dismounted from the aluminum double-sided tape mount and transferred to the scanning transmission electron microscope (STEM, FEI Titan 80-300 operated at 300kV), for atomic resolution imaging of the organic matter – mineral associations, and selected area diffraction (SAD). This step, however, especially the focused electron beam during the SAD acquisition, produced some rad damage and C deposition on scanned areas. Energy dispersive spectrometry (EDS) coupled to the STEM was also performed, but due to the induced radiation damage and C deposition, as the very last step.

Overall, this labor-intensive correlative procedure brought valuable insight into the soil microbes and soil organic matter association with minerals, with important implications on the soil organic carbon stabilization.

In the near future, a machine learning (ML)-enabled recognition of microbial features will be a profound advantage in identifying, recording and statistically accounting the ROI [4].

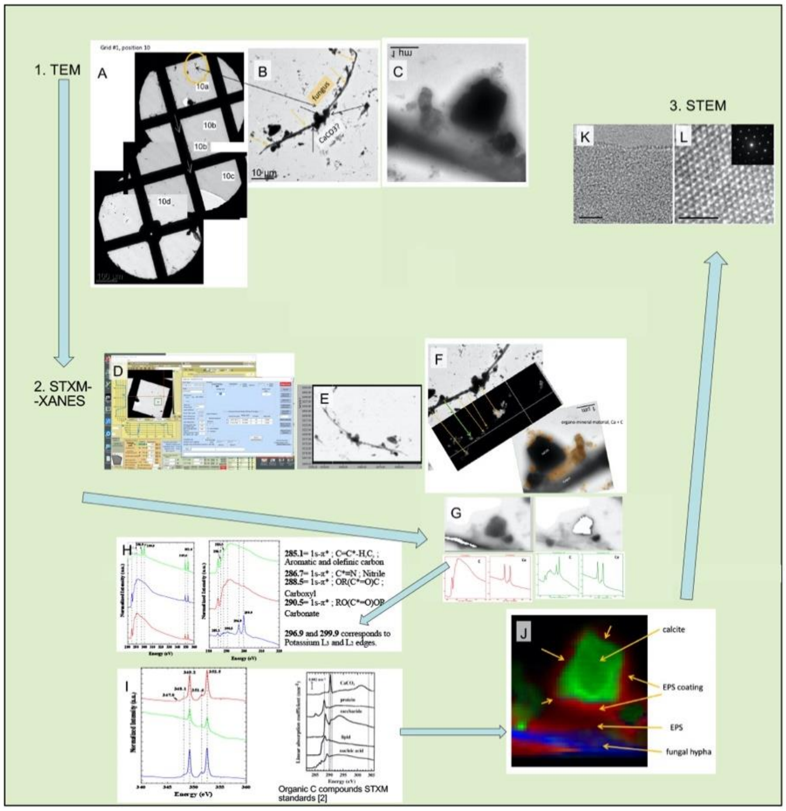

Figure 1. Three main steps in spatially correlated electron and X-ray high resolution chemical imaging method. 1.) ROI are located on a grid using a low dose TEM imaging (A; a higher magnification - inset B, C). A map is constructed. 2.) The grid is transferred to the STXM instrument. The mapped positions are used to locate the ROI (D, E, F, G). XANES spectra are collected from ROI (H, I). (j) - an analyzed spectral map. 3.) The grid is transferred to the STEM instrument, for atomic resolution imaging of the crystalline phases, and SAD (K, L).

References:

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