

## Elemental Co-localization of Nutrients, C, Al, and Fe in Soil Minerals with Electron Microscopy and Scatterplot-matrix Analysis

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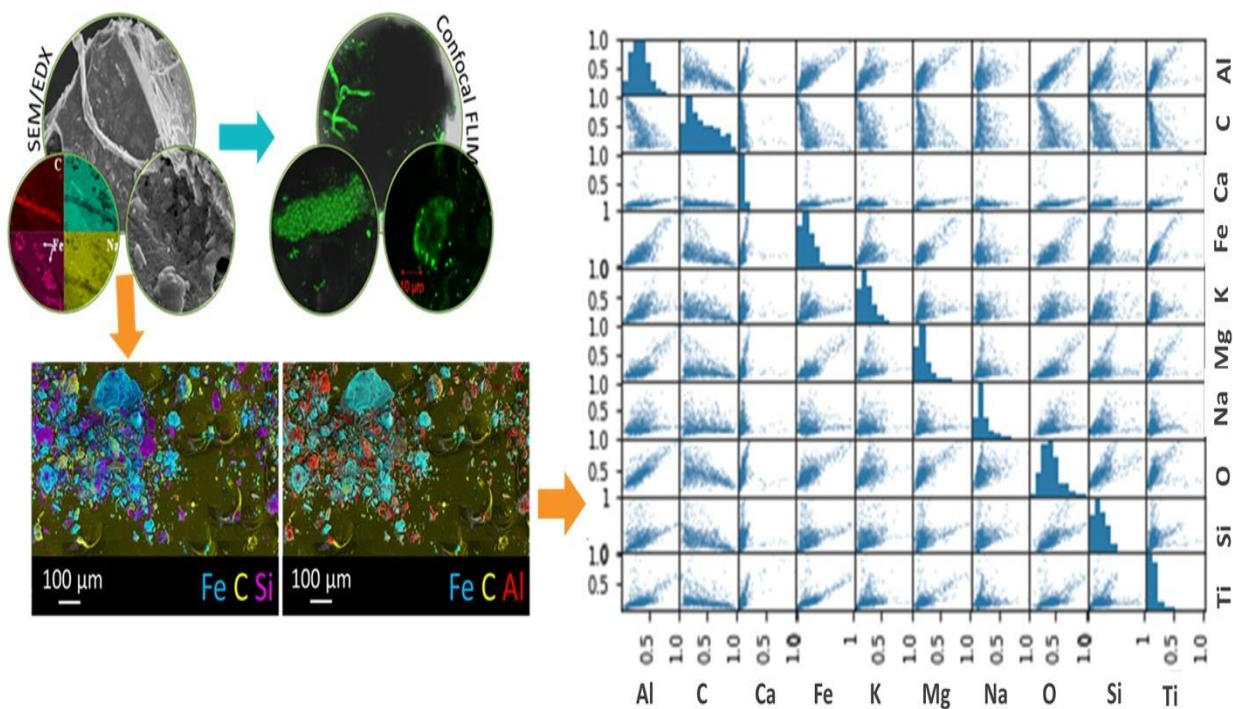
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Transformation of minerals on Earth's surface is an important process because it supplies terrestrial ecosystems with essential nutrients that are vital for the life of soil microbes and for plant growth. In addition, mineral transformation influences organic carbon capture as well as sequestration of atmospheric carbon dioxide (CO<sub>2</sub>) in soils, with global impact towards reduction of CO<sub>2</sub> levels in atmosphere. Understanding linkages between mineral transformation, nutrient availability, and C capture in varying ecosystems can advance our knowledge on ecosystems responses to a warming climate and can guide in developing more accurate models to mitigate climate change.

The focus of this research is i) to gather micron to mm-scale insights on distribution of essential nutrients and C balance in soils from understudied ecosystems and ii) to interrogate the impact of bedrock on C-storage on these soils. Specifically, here we investigate the soils developed at cold wet climates of the northern latitudes, i.e. coastal temperate ecosystems. It is known that these ecosystems store significant amounts of soil C, a process that is driven by high biomass productivity during wet and warmer summer temperatures followed by cool and slow C decomposition rate during winter season. Here we study the association between minerals, nutrients, and the organic matter, which includes microbes and decaying organic material in the soil matrix, by using scanning electron microscopy (SEM) analytical tools and scatter-plot matrix analysis. To further investigate organic matter association with minerals, we combined confocal microscopy with SEM images which assessed bacterial and fungal-mineral interactions.

Prior to analysis the soil samples were dried under a flow of N<sub>2</sub> to preserve their oxidation state. The dry soil samples that contain mineral grains ranging from sub- $\mu\text{m}$  to 100- $\mu\text{m}$ 's were fixed and secured into Al-stubs with C- or Cu-based sticky tape. The SEM (Helios 600i) and energy dispersive x-ray spectroscopy (EDX, Aztec\*, Oxford Inc.) equipped with automatic Large Area Mapping analysis tool was used to collect elemental compositional maps. In one automated analysis, we collected images composed up to 100's fields of view, which allowed construction of mm-scale compositional elemental maps per each soil sample as well as interrogation of selected areas of interest, at higher sub macro-scale resolution. The elemental maps revealed evident differences between the type of minerals, nutrients, and organic C (Fig 1). However, to aid our understanding of the degree of correlations between the elements of interest and mineral composition, we developed a custom python script for pixel-by-pixel correlation analysis and using this script we analyzed the compositional maps (Fig. 1). We used the oxygen-depleted EDX maps to generate a background mask which was then applied to all the EDX images. For correlation analysis, a binning factor of 100 was used to group pixels together and to reduce noise. The scatter matrices and scatter plots were generated afterwards using Python Data Analysis Library (Pandas) (Fig. 1). The combination of microscopy and matrix analysis reveals clear evidence of correlation between Al- and Fe- bearing minerals and nutrients in soil and when combined with confocal microscopy reveals co-localization of organic C with soil minerals. Such coupling of analytical tools with statistical methods in particular is useful for studying complex systems, such as the soil matrix, where materials and interfaces frequently

overlapping signals.



**Figure 1.** Electron microscopy and confocal microscopy showing colocalization of organic matter with soil minerals. Energy Dispersive X-ray Spectroscopy Large Area Maps were used to generate scatter matrices and scatter plots (shown here) that reveal correlation of nutrients with minerals in soil.