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Laser scanning confocal microscopy – soil particle fluorescence

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Soil organic matter (SOM) coatings on soil particles, in their natural state, are thought to be responsible for some bulk sample properties such as wettability. This material is difficult to study *in-situ* under normal environmental conditions.

Laser Scanning Confocal Microscopy (LSCM) is used in a wide range of biological investigations, where either the fluorescence of introduced fluorophores or auto-fluorescence of samples can be used to obtain images of specimens. The high resolution of this technique allows samples of the size of a single cell to be depicted in reasonable detail and scanning through a sequence of focal planes can give information about the 3D structure of a sample.

Auto-fluorescence occurs in many natural substances due to the complex molecular structure of any components with large conjugated π-(electron bonding) systems. Humic acids are ubiquitous components of SOM and show auto-fluorescence over a wide range of excitation wavelengths (Hansen & Schnitzer, 1968). Preliminary tests also showed that soil particles generally auto-fluorescence over a wide range of wavelengths with a spectral pattern dependent upon the nature of the laser source.

Sample soil particles were individually mounted on glass cover slips, using waterproof cyanoacrylate adhesive, and covered with a removable second one held out of contact with the particle. This assembly allowed repeated examination of particles in exactly the same position to facilitate tests of repeatability and reproducibility of particle fluorescence.

Fluorescence measurements were made using a LSCM (model LSM 510 Meta, Zeiss, Germany). Particles were imaged using irradiation wavelengths of λ= 488 nm and λ = 543 nm and the fluorescence emitted was quantified. At least 15 particles of $\sim<$ 200μm were randomly selected from various sandy soil samples possessing various organic carbon contents and exhibiting various degrees of water repellency.

Particles were also subjected to discrete fluorescent spectral analysis (DFSA). This method provides images associated with the emission over discrete wavebands. A series of such wavebands allows a more detailed examination of the emission spectrum of the particle. DFSA may give information about differences in the nature and distribution of organic material on a particle and between different particles.

Results suggest that LSCM is a reliable tool in detecting SOM coatings on soil particles and some good correlations between bulk soil total organic carbon content and soil particle fluorescence have been obtained. Although these maybe somewhat fortuitous the techniques provides a potential tool for detailed examination of the response of individual particle surfaces to various treatments that may assist in elucidating its in-situ nature and behaviour.