Integrating physical and chemical techniques to characterise soil microsites.

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Abstract

Many problems in environmental and soil research require techniques that quantify the soil micro-environment. It has become increasingly apparent that we need novel micro-analytical techniques to compliment well established methods that study soils at macro-spatial scales. Despite tremendous progress in this field over the last decade, quantitative methods have developed within separate disciplines and operate at different spatial scales hampering their integration. Moreover, it means that opportunities to integrate these methods may be overlooked. In this paper we develop methods that enable integration of a 3-D non-invasive technique to characterise soil structure (X-ray CT) with a 2-D spectroscopic method that characterises the spatial distribution of chemical elements on surfaces (SEM-EDX). First we developed statistical software to locate the 2-D plane in which SEM-EDX analyses were performed within the 3-D volume. Then we demonstrate that selected compounds, including particulate sources of C and $CaCO_3$, can be quantified with SEM-EDX and subsequently visualized within the 3-D soil environment. Finally, we demonstrated that when we characterized sequential slices with SEM-EDX we could use co-kriging methods to predict the 3-D spatial distribution of chemical elements. We discuss the possibilities and problems that need to be resolved to combine these methods.

Key Words

X-ray tomography, soil-micro habitat, scanning electron microscope, co-kriging. modelling.

Introduction

Increasingly, over the last few years, it has become clear that our measurements in soils are made at scales too coarse to allow for precise prediction of ecosystem processes. Fortunately, recent technological advances allow us to unravel the physical, chemical, and biological heterogeneity of soils, which, when combined with modelling techniques, allow us to make sense of the complexity of soil systems. However, separation of disciplines still hampers progress. Soil physics and soil chemistry are highly interdependent, with the heterogeneous spatial distribution of chemical species intertwined with soil structure. Examples include the role of metal complexes and clay minerals in the formation and stabilization of aggregates (Tisdall and Oades 1982), gleyic features in poorly drained soils, or soils contaminated with metals. Correlations between soil structure and chemical properties also have significant impact on macro-pore flow of solutes (Ellerbrock *et al*. 2009).

To date, our understanding of soil structure has relied heavily on the concept of soil aggregates. Advances in the use of X-ray CT, however, enable quantification of the internal structure of soils at microscopic scales without physical disruption. Similarly, microscopic and micro-spectroscopic analyses (soft tissue X-ray microscopy and near edge X-ray absorption fine structure) are beginning to increase the spatial resolution of chemical analysis. However, these chemical techniques to date are restricted to small samples (a few mm in diameter) and often require access to synchrotron facilities, even though the development of tabletop X-ray CT/ micro XRF scanners is making rapid progress (Sasov *et al*. 2008). Combining non-invasive and invasive techniques that operate at microscopic scales may offer a way beyond what can be achieved with current techniques.

Despite the overwhelming evidence of close links between chemical composition and soil structure, techniques for quantification are often separate, and it may be a few years before 3-D mapping of the physical, chemical and biological properties in soils is possible at a range of spatial scales. In this paper we investigate if we can combine 3-D X-ray CT with semi-quantitative element maps obtained with 2-D spectroscopic methods (SEM-EDX) to unravel the interactions between soil physical and chemical characteristics. The voxel greyscale value in a 3-D X-ray CT reconstruction represents the linear attenuation coefficient, which is a function of the atomic number detected by SEM-EDX, hence the potential for integration of these techniques. The overarching aim is to explore and develop methods to combine 2-D chemical analyses with 3-D physical data. We aim to achieve this through the following specific objectives: **1.** to use X-ray tomography and identify organic and mineral compounds that differ sufficiently in attenuation to be visualized in 3-D. **2.** to produce parallel 2-D slices through soil and analyze these with SEM-EDX. **3.** to apply and test statistical methods to interpolate between the parallel slices and produce a 3D map that visualises the distribution of the 3-D soil structure.

Methods

Sample preparation and procedure

A sandy loam soil was air-dried, sieved to 1-2 mm and repacked at a bulk-density of 1.2 Mg/m^3. Blocks were resin impregnated and precision-sliced to produce a 1 cm^3 soil cube. The physical structure was quantified using X-ray CT (see below). After that, the cube was sliced to produce parallel slices of about 500 µm thick, and the slices that were scanned on both sides with SEM-EDX to identify both mineral and organic components.

SEM-EDX

We used the SEM-EDX system (Jeol 6460LV and Oxford Instruments InCA X-Sight) to quantify and map elemental spatial distributions on the surfaces of slices from a resin-impregnated 1 cm^3 of soil. Element maps were acquired at 15 kV with a spatial resolution of 8 µm. External elemental and mineral standards were used to ensure data quality control during data acquisition. Phase analysis was used to identify distinctive chemical compounds based on their chemistry. Phase analysis, was also used to isolate the impregnating resin's chemistry from the soil data.

X-ray micro-tomography

The 3-dimensional pore space was visualised with a METRIS HMX micro-tomography system at 8 µm resolution. High resolution CT data sets were reconstructed in 3-D using floating point grey scale to enhance contrast between the phases. We used in-house developed software for a quantitative analysis, segmentation, and visualization of the 3-D pore structure and solid phases.

Analytical methods: Combining X-ray CT and SEM-EDX

We explored two ways of combining the techniques: (1) use SEM-EDX to characterise compounds that differ in attenuation in X-ray CT, so that they subsequently can be visualized in 3-D and (2) use co-kriging to predict the 3-D distribution having located the sequential slices within a 3-D volume. In the first method we identified compounds in the X-ray CT data with an attenuation coefficient significantly different from the surrounding soil matrix (Young *et al*. 2009). Typically these include particulate sources of organic matter (e.g. black carbon), horneblends, or $CaCO_3$.We segmented out these identifiable objects using in-house developed thresholding software and algorithms, and then obtained a thin section in which the chemical composition is quantified using SEM-EDX. In the second method, we first aligned the spatial resolution, and then used spatial cross-variograms to locate the 2-D SEM-EDX plane within the 3-D physical structure. A close (µm's) alignment is essential due to the huge microscopic heterogeneity. Based on the spatial correlation between the physical and chemical data we then applied ordinary co-kriging to predict the 3-D distribution of chemical elements.

Results

Using SEM-EDX it was possible to obtain detailed information on the spatial distribution of chemical elements for a relatively large sample area (1 cm^2). We produced spatial maps of several elements including C, O, K, Ca, and Si, which demonstrated the high spatial variability of those elements at small scales (Figure 1). The data showed a good spatial correlation between the physical structure and the concentration of some elements such as carbon which was mainly occupying the pore space as it is associated with the resin used to impregnate the soil sample, silicon that was present mostly on the solid phases, and calcium-carbonate that was associated with very large greyscale values in the X-ray CT images.

Some chemical elements had attenuation coefficients in the X-ray CT data sufficiently different from the background to allow for segmentation (Figure 2). For example, the light 'kidney-shaped' object in the X-ray CT data set (Figure 2a) was quantified by SEM-EDX as $CaCO_3$ (Figure 2b), evidenced by a quantitative analysis of the composition of this object as C = 22.73%, O = 39.5%, Ca = 37.1%, and Si, Al, and Mg <0.5 % on a weight basis. Further analysis of the X-ray CT data enabled us to visualize this compound, now identified as $CaCO_3$, in 3-D within the soil volume (Figure 2c).

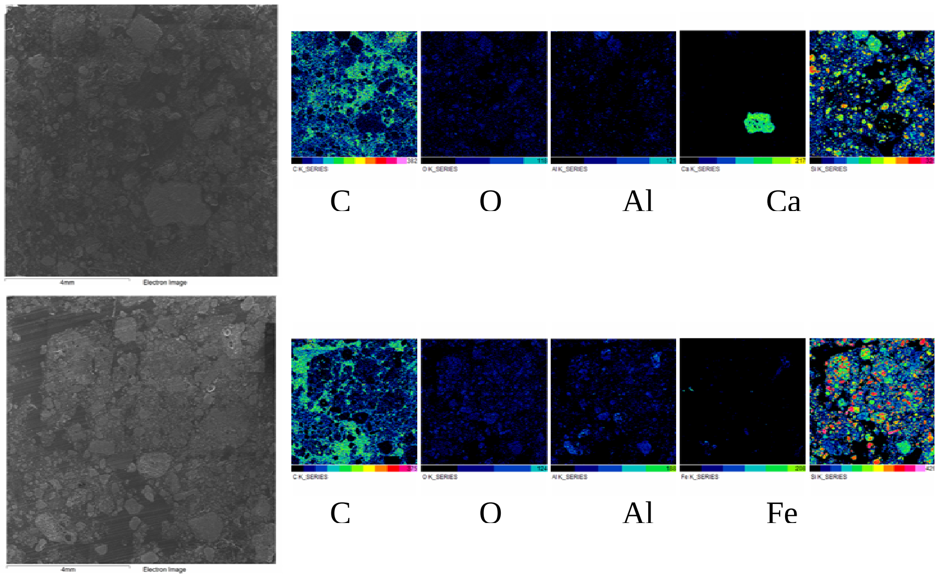
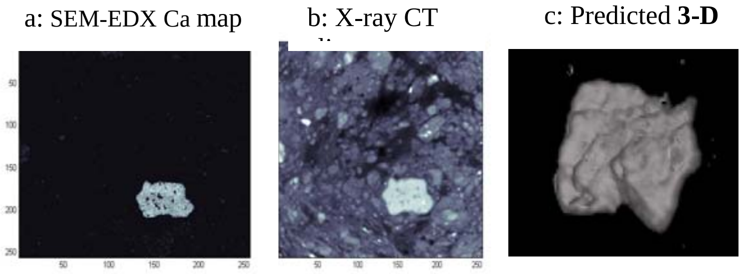


Figure 1. SEM image of the two 1 cm^2 faces of a 500 µm thick soil slice, and the spatial distribution of C, O, K, Ca, Fe, and Si within these slices quantified at an 8 µm resolution.


a: SEM-EDX Ca map

b: X-ray CT

c: Predicted **3-D**

Figure 2. A spatial map obtained with SEM-EDX for (a) Ca, the same slice within the 3-D data obtained with X-ray CT (b), and segmentation of $CaCO_3$ from the X-ray CT data (c).

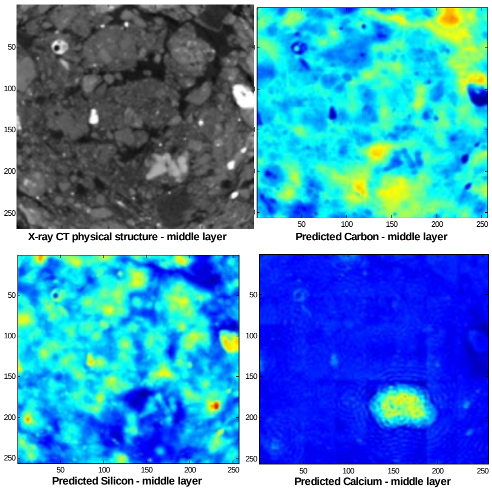


Figure 3. An example of the use of co-kriging to predict the spatial distribution of C (top right), Silicon (left bottom) and Ca (right bottom) in the plane half-way between the two layers that were analysed with SEM-EDX. The figure top left is the X-ray CT slice of the same layer. The figure shows that even with only 2 layers in which chemical data are available a reasonable prediction in 3-D can be made.

Based on the spatial correlation between the physical and chemical variables, we applied an interpolation method using ordinary co-kriging, to predict the internal 3D chemical structure. The predicted chemistry showed a good agreement with the physical structure as indicated by the co-dispersion coefficient between the two variables. In particular, the predicted carbon concentration remained consistent with the pore space,

(Figure 3), while silicon was present in soil aggregates. In this initial stage the statistical results look promising and further investigation including method development and data acquisition for model validation will be undertaken to improve the prediction of 3D chemical structure.

Conclusion

To forward our understanding of soil ecosystems it is imperative that we advance our current techniques to obtain a characterization of the soil micro-environment. Major advances have been made recently for the physical characterization of soil, but 3-D insight into the distribution of chemicals and microbes remains challenging. In this paper we demonstrated that by combining techniques and using statistical models it is possible to obtain a 3-D quantification and visualization of the chemical heterogeneity at microscopic scales. We tested two methods to combine X-ray CT and SEM-EDX. In the first method we identified objects that can be distinguished with X-ray CT and subsequently quantified and chemically characterized them with SEM-EDX. We demonstrated how this can be applied to CaCO3 in our soil sample. This method is however restricted to a limited number of compounds but promising compounds include hornblends and particulate organic matter, including black carbon. This does enable to isolate fractions and visualize them within a 3-D environment, though the full range of options requires further investigation in particular if the material is more heterogeneous by nature.

The SEM-EDX application is restricted to 2-D surfaces of soils. However, by taking sequential slices a 3-D visualization of the chemical heterogeneity can be obtained. It is however useful to be able to reduce the number of slices that is required for extrapolation to 3-D, as characterization of 2-D surfaces with SEM-EDX is time consuming, and because some material gets lost when preparing sequential slices through a soil sample. The agreement between X-ray CT and SEM-EDX did allow us to test if co-kriging methods can be used to interpolate chemical data between two sequential 2-D layers. Co-kriging by combining X-ray Ct and SEM-EDX significantly reduced the number of slices that is required to predict the 3-D chemical heterogeneity. The predicted chemicals were in good agreement with the physical structure as indicated by the co-dispersion coefficient between the two variable. This was in particular the case for carbon and silicon, with carbon predominantly associated with the pore space (resin of the impregnated blocks) and silicon with the solid phase. Further investigation is however required to improve the statistical techniques and to optimize the sampling strategy to obtain the best possible prediction.

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