SPATIALLY RESOLVED QUANTIFICATION BY NANOSIMS OF ORGANIC MATTER SORBED TO (CLAY) MINERALS

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Soils are highly heterogeneous entities in which organic and inorganic as well as living and non-living building blocks interact to form biogeochemical interfaces. While processes at these interfaces occur at the micrometer or submicrometer scale, they are thought to influence the behavior of soils at the global scale, *e.g.* soils as carbon sinks. Analytical methodologies with a high resolution are, therefore, required in order to investigate these processes with the final goal to understand biogeochemical-interface formation mechanistically. In the present study, sorption experiments of water-extractable organic matter on model minerals, such as boehmite and illite, were performed. Adsorption of organic matter on the minerals was quantified by conventional bulk-scale methods and compared with data from nanoSIMS measurements. From the data obtained, scaling factors have been developed which permit the quantification of organic matter in the secondary ion images provided by nanoSIMS.

1. Introduction

Soil structure results from soil-forming processes at the molecular scale, and this determines the behavior of soils not only at the macroscopic but also at the global scale. In this context, soil organic matter (SOM) is important not only as a carbon sink but also as a gluing agent for soil structure. The mechanisms behind the formation of soil aggregates remain a matter of some debate in soil science (*e.g.* Six *et al.,* 2000; Lehmann *et al.,* 2007). While earlier work on these issues was based mostly on conventional bulk analyses, imaging techniques which combine spatial and chemical information provide additional information on the distribution of SOM at a more relevant scale.

Bulk-scale analyses allow quantification of SOM within the soil structure along with qualitative data on the predominant chemical binding types. All information on the spatial distribution of SOM at the submicron scale is lost during these analyses, however. While qualitative data on the spatial distribution of SOM can be recorded with nanoscale secondary ion mass spectrometry (NanoSIMS) (*e.g.* Mueller

et al., 2012; Vogel *et al.*, 2014), quantification of SOM at the microscale remains challenging due to matrix and charging effects. The present study aimed to develop scaling factors from bulk-scale methods to NanoSIMS data, in order to approach the challenge of SOM quantification at the submicron scale.

2. Materials and methods

2.1. Sample material

In order to produce relatively simple test systems, model minerals were supplied with a defined amount of organic matter in sorption experiments. Organic material was harvested from the 7 cm-thick superficial organic layer of a podzol near Jena, Germany (50.911937°N, 11.631375°E). After drying at 20°C in dark conditions, the organic material was passed through a 2 mm sieve. Water-soluble organic matter was extracted from this material according to the protocol of Kaiser *et al.* (1996). Briefly, the organic material was mixed with deionized water (final concentration = 300 g_{dw} L^{-1}) by stirring for 15 min. For the following 24 h the suspension was incubated in the dark in order to extract the water-soluble organic material. After the incubation, the suspension was passed through a 0.45 μm PVDF (polyvinylidene fluoride) filter to separate the water-soluble organic material. This stock solution was stored for no more than 3 days at 5°C in the dark prior to use.

Illite and boehmite were chosen as model minerals. Illite was obtained from Inter-ILI Engineering Co. Ltd. (Kosd, Hungary) and boehmite (Apyral® AOH 20) from Nabaltec AG (Schwandorf, Germany). The N_2-BET surface areas of the minerals were measured using an Autosorb-1 analyser (Quantachrome, Syosset, NY, USA) and amounted to 43.8 m^2 g^{-1} for illite and 2.8 m^2 g^{-1} for boehmite, respectively.

The minerals were loaded with six different concentrations of organic material in triplicate sorption experiments. For these experiments the stock solution was diluted by means of deionized water as follows: 1:1, 1:2, 1:5, 1:10, 1:50, 1:100. The pH was adjusted to 6.5 for all dilutions. Minerals were suspended in the dilutions with a final concentration of 50 $g_{mineral}$ L^{-1}. Experiments were performed in the dark at 20°C in an overhead shaker at 32 rpm for 5 days. After the experiments, the minerals and the water-soluble organic-matter solution were separated by means of centrifugation at 537 × *g*. The solid phase was freeze dried immediately thereafter, while the liquid phase was prepared immediately for further analysis.

2.2. Bulk analyses

To measure carbon and nitrogen concentrations in the solid phase, ∼30 mg_{dw} of sample was weighed into tin containers which were combusted in a C/N analyser (Euro EA – CHNSO elemental analyser, Hekatech, Wegberg, Germany). Measurements of the carbon concentration of the liquid phase required dilution of the samples below 25 $μg_C$ L^{-1} and acidification by means of 2 drops of 2 mol L^{-1} HCl. Analysis was performed by means of a TOC 5050 A analyser (Shimadzu, Kyoto, Japan).

2.3. SEM and NanoSIMS analyses

Solid samples for NanoSIMS analyses were prepared in accordance with the method of Vogel *et al.* (2014). Briefly, freeze-dried solid samples were re-suspended in deionized water (final concentration = 0.1 g_{dw} L^{-1}) and distributed onto a silicon wafer in the form of a 2 μL drop and left to dry in a desiccator. Prior to analysis, the samples were coated with a ∼5 nm gold layer by means of a sputter coater (SCD 005, Baltec, Balzers, Liechtenstein).

Subsequently, five individual particles, serving as regions of interest (ROIs) for Nano-SIMS analyses, were selected randomly using a scanning electron microscope (SEM; JSM 5900 LV, Jeol, Eching, Germany). The coordinates of the ROIs were converted to the coordinate system of the NanoSIMS using the *simscoord* software (www.soil-science.com/nanosims; programmed by Johann Lugmeier).

The cesium source of the NanoSIMS with an ion-beam current of 18.545 nA was used for analysis and the mass resolving power was adjusted in order to resolve reliably the isobaric interferences observed. The fields of view ranged from 20 μm × 20 μm to 30 μm × 30 μm and contained 256 × 256 pixels with a dwell time of 30 ms $pixel^{-1}$. The aperture of the instrument was adjusted in order to prevent saturation of the mass spectrometers: D1_3, ES_3, AS_0, EnS_0. The sample surface was pre-sputtered with a high-current Cs^+-beam for 5 min prior to the measurements, in order to remove the conductive gold layer, to reach stable count rates, and to increase the yield of secondary ions for analysis. The selected masses were chosen to detect organic matter ($^{12}C^-$, $^{12}C^{14}N^-$, $^{32}S^-$) along with the model minerals ($^{16}O^-$, $^{28}Si^-$, $^{27}Al^{16}O^-$). The NanoSIMS data were analysed by means of the *Image J* plugin *openmims* (Poczatek *et al.,* 2009).

2.4. Data analysis: scaling factors

The experiment presented was designed to generate minerals carrying various amounts of organic matter. Bulk-scale analysis of this material yielded the exact carbon concentration in the minerals at the various concentration steps. These data allowed the construction of Freundlich-type adsorption models.

NanoSIMS analysis of the surfaces of mineral particles in the six concentration steps provided spatially resolved count rates for the various ion species detected. Because the count rates for organic matter show large variations, and thereby prevent statistically sound relationships, the surface coverage of the minerals with organic matter was analyzed in a similar way to that of Vogel *et al.* (2014). The threshold tool (default mode) of the *openmims* software (based on the isodata algorithm of Ridler and Calvard, 1978) determined a threshold above which the minerals were stated to be covered with organic matter. The fraction of organic matter-covered pixels thus defined the organic-matter surface coverage (OMSC) of an ROI in the NanoSIMS data.

The scaling factors developed in the present study thus represent the relationships (fits) found between the measured carbon concentrations (bulk methods) and the OMSCs derived from the NanoSIMS data. Correction for edge effects was necessary

because during NanoSIMS analysis edges led to larger count rates for ion species representative of organic matter ($^{12}C^-$, $^{12}C^{14}N^-$, $^{32}S^-$). Smaller particles, thus, have greater edge-to-surface area ratios when projected onto a 2D-view compared to larger particles. Normalizing the OMSC data for the particle size of each analyzed ROI accounted for this effect and the data derived were reported as cOMSC (corrected OMSC) values.

3. Results and discussion

The carbon and nitrogen concentrations measured at the bulk scale were comparable for the liquid and solid phases. The data measured were described by Freundlich adsorption models with comparable concentrations adsorbed onto illite and boehmite (Figure 1). Besides, illite had a surface area which was 15 times greater; thus, boehmite adsorbed more organic matter per unit surface area.

The spatially explicit analysis of the surface coverage of organic matter in the Nano-SIMS data of illite and boehmite provided greater OMSC in higher-concentration steps. As shown in Figure 2, however, greater count rates were recorded at the edges of the minerals, while the centers of the particles showed lesser counts. The reason for this effect could either be charging, which was accounted for by means of the electron-flood gun of the NanoSIMS instrument, or that the organic layer on the mineral particles was analyzed vertically at the edges of the minerals rather than horizontally as at the centers of the particles.

The visible intensity gradient for the silicon wafer across the NanoSIMS image in Figure 2 might also be a result of charging. As the background was excluded from the OMSC analysis, however, it is probably irrelevant to the derived results.
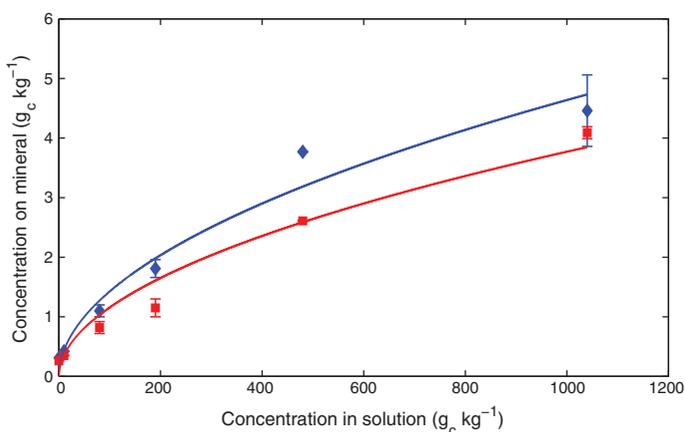

Figure 1. Freundlich adsorption isotherms for adsorption of organic matter onto illite (blue line, ◆) and boehmite (red line, ■).

As indicated by representative NanoSIMS images (Figure 3), more counts were achieved with $^{12}C^-$ in the experiments with illite, while $^{12}C^{14}N^-$ was a superior indicator of SOM in the experiments with boehmite. Derived from all NanoSIMS images of the largest organic-matter concentration experiment, mean counts for illite were 32.4 $^{12}C^-$ counts pixel^{-1}, but only 0.35 $^{12}C^{14}N^-$ counts pixel^{-1}. For boehmite, the discrepancy was reversed, but less pronounced, with 30.13 $^{12}C^-$ counts pixel^{-1} *vs.* 86.30 $^{12}C^{14}N^-$ counts pixel^{-1}. This matrix effect was accounted for in subsequent data analysis, using $^{12}C^-$ as the indicator of organic matter on illite particles and $^{12}C^{14}N^-$ as the indicator of organic matter on boehmite particles.

Figure 2. Overlay SEM image and a NanoSIMS ^{12}C ion micrograph of the same illite particle, indicating greater count rates at the edges of the mineral particles.

Comparative analysis of the data from NanoSIMS measurements with those from bulk-scale methods yielded linear relationships between the carbon content measured by C/N analyses and the $^{12}C^-$ (illite)- or $^{12}C^{14}N^-$ (boehmite)-derived OMSCs calculated from the NanoSIMS data. The quality of these linear fits was further improved when the NanoSIMS data were normalized for edge effects on the particles (cOMSC) (Table 1). Quantification of organic matter in these relatively simple systems may, therefore, be possible.

For more accurate measurements, other processes need to be investigated by more of the type of data analysis presented here. For example, topographical effects are currently ignored but can have serious impacts on the accuracy of the NanoSIMS measurements (*e.g.* Kita *et al.,* 2009) even when analysing ion ratios. This issue can only be resolved by a combination of NanoSIMS with surface-sensitive techniques working at the same resolution, *e.g.* atomic force microscopy (Rennert *et al.,* 2012).

4. Conclusions

NanoSIMS provides unrivalled insights into the microstructure of soils, sediments, and organo-mineral associations. The technique typically fails to provide quantitative data
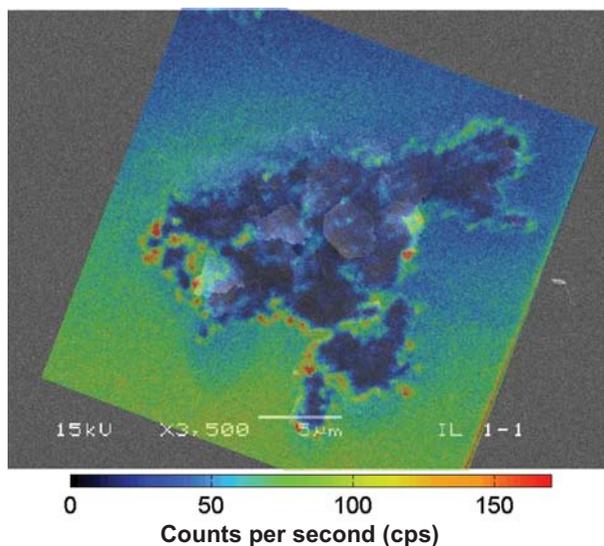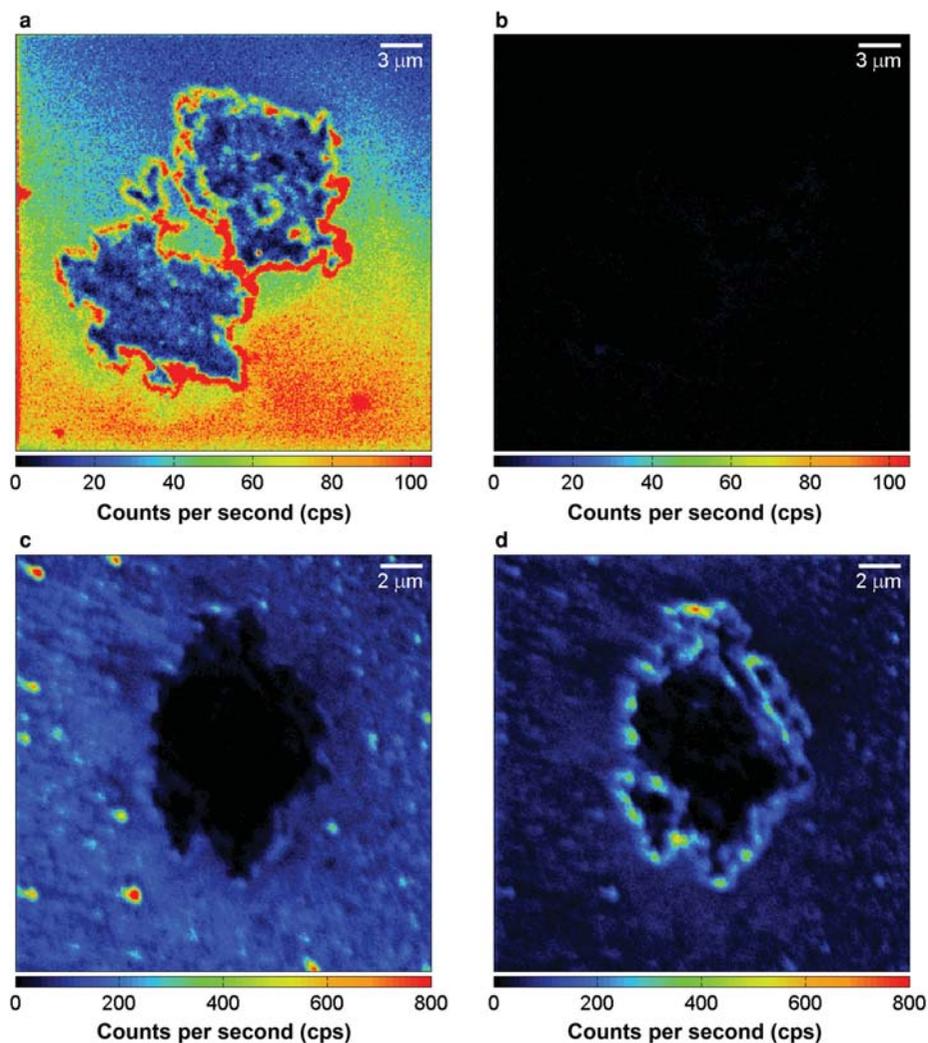
Figure 3. Representative NanoSIMS images for organic material on mineral particles of illite (a: $^{12}C^-$ ions, b: $^{12}C^{14}N^-$ ions) and boehmite (c: $^{12}C^-$ ions, d: $^{12}C^{14}N^-$ ions).

on the concentration of certain ion species at the submicron scale. The scaling factors developed in this study now allow the matrix effects of various minerals to be taken into account when determining organic matter content. An improved understanding of the quantity of SOM adsorbed to various surfaces in soil will be possible by finding similar relationships with other soil constituents. Contrary to the bulk-scale methods, the spatial information and resolution is preserved by means of this new approach.

Table 1. Comparison of the bulk-scale concentrations, organic matter surface coverage (OMSC), and corrected organic matter surface coverage (cOMSC) in the NanoSIMS data-allowed definition of linear relationships. The correlation coefficients (r^2) shown were computed from the data, rather than from the fit. Reported standard deviations originated from $n = 5$ replicates.

Mineral	C concentration (g_c kg^{-1})	OMSC (m^2 m^{-2})	Scaling factor	cOMSC (m^2 m^{-2})	Scaling factor
Illite	0.31	0.06 ± 0.02	$y = 0.0206x$	0.07 ± 0.03	$y = 0.0302x$
	0.42	0.12 ± 0.05	$+ 0.101$	0.14 ± 0.06	$+ 0.112$
	1.10	0.19 ± 0.05		0.18 ± 0.04	
	1.81	0.14 ± 0.05	$r^2 = 0.61$	0.19 ± 0.02	$r^2 = 0.81$
	3.77	0.13 ± 0.04		0.18 ± 0.02	
	4.46	0.22 ± 0.04		0.27 ± 0.07	
Boehmite	0.26	0.17 ± 0.07	$y = 0.0387x$	0.16 ± 0.04	$y = 0.0392x$
	0.35	0.09 ± 0.05	$+ 0.104$	0.12 ± 0.06	$+ 0.149$
	0.82	0.10 ± 0.06		0.10 ± 0.05	
	1.15	0.18 ± 0.09	$r^2 = 0.81$	0.21 ± 0.11	$r^2 = 0.82$
	2.61	0.16 ± 0.04		0.17 ± 0.04	
	4.09	0.29 ± 0.07		0.31 ± 0.08	

Acknowledgments

References

Kaiser, K., Guggenberger, G., and Zech, W. (1996) Sorption of DOM and DOM fractions to forest soils. *Geoderma*, **74**, 281–303.

Kita, N.T., Ushikubo, T., Fu, B., and Valley, J.W. (2009) High precision SIMS oxygen isotope analysis and the effect of sample topography. *Chemical Geology*, **264**, 43–57.

Lehmann, J., Kinyangi, J., and Solomon, D. (2007) Organic matter stabilization in soil microaggregates: implications from spatial heterogeneity of organic carbon contents and carbon forms. *Biogeochemistry*, **85**, 45–57.

Mueller, C.W., Kölbl, A., Hoeschen, C., Hillion, F., Heister, K., Herrmann, A.M., and Kögel-Knabner, I. (2012) Submirocn scale imaging of soil organic matter dynamics using NanoSIMS – from single particles to intact aggregates. *Organic Geochemistry*, **42**, 1476–1488.

Poczatek, C., Kauman, Z., and Lechene, C.P. (2009) *OpenMIMS ImageJ* plugin Guide. Harvard Medical School, Boston, Massachusetts, USA.

Rennert, T., Totsche, K.-U., Heister, K., Kersten, M., and Thieme, J. (2012) Advanced spectroscopic, microscopic, and tomographic characterization techniques to study biogeochemical interfaces in soil. *Journal of Soils and Sediments*, **12**, 3–23.

Ridler, T.W. and Calvard, S. (1978) Picture thresholding using an iterative method. *IEEE Transactions on Systems, Man, and Cybernetics*, **8**, 630–632.

Six, J., Elliott, E.T., and Paustian, K. (2000) Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biology & Biochemistry*, **32**, 2099–2103.

Vogel, C., Mueller, C.W., Höschen, C., Buegger, F., Heister, K., Schulz, S., Schloter, M., and Kögel-Knabner, I. (2014) Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils. *Nature Communications*, **5**, 2947.