Elucidating soil structural associations of organic material with nano-scale secondary ion mass spectrometry (NanoSIMS)

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Abstract
The specific features of the nano-scale secondary ion mass spectrometry (nanoSIMS) technology with the simultaneous analysis of up to seven ions species with high sensitivity and resolution enables us to perform multi-element and isotope measurements at the submicron-scale. In order to demonstrate the power of this technique, we performed an incubation experiment with primary soil particles of the fine silt and clay fraction and soil aggregates (< 6.3 µm) from an Albic Luvisol and a Haplic Chernozem, respectively, with a ^{13}C and ^{15}N labelled amino acid mixture as tracer. Before and at selected time intervals after addition of the tracer, samples were derived and prepared for nanoSIMS investigation. For this purpose, different sample pre-treatments for single soil particles and soil aggregates were developed. Primary soil particles high in carbon showed an enrichment of ^{13}C and ^{15}N after label addition which decreases over time. In soil aggregates, nanoSIMS analyses revealed that the labelled amino acids were transported most likely by diffusion through the pores of the aggregate from the outside to its interior. From these first results, it can be concluded that the nanoSIMS technology will allow a major step forward in the understanding of biogeochemical processes and properties of soil.

Key Words
^{13}C, ^{15}N, aggregate, label, stable isotope, submicron-scale.

Introduction
Soils consist of a complex mixture of solid, liquid and gaseous components. Major inorganic solid components are quartz, clay minerals, oxides and hydroxides of Fe, Mn and Al and carbonates. Soil organic matter (SOM) represents a complex mixture of partially recalcitrant substances composed of humified and non-humified materials derived from plant litter, faunal and microbial biomass, like e.g. polysaccharides, lignin, aliphatic biopolymers, tannins, lipids, proteins and amino sugars. Accordingly, soils are structurally heterogeneous across a wide range of spatial and temporal scales (Herrmann *et al.* 2007b; Totsche *et al.* 2010). During soil formation, primary soil particles are rearranged and glued together to microaggregates which are bound together to macroaggregates. Subsequently, a hierarchic aggregate system of increasing structural and functional complexity is formed (Totsche *et al.* 2010). Microorganisms inhabit the thus developed microhabitats and change them according to their own needs (Herrmann *et al.* 2007a, b). In order to unravel the heterogeneous composition of these submicron sized organo-mineral associations, the simultaneous analysis of the spatial distribution of C, N and other elements at the nano-scale will allow a major step forward in the understanding of soil formation with significant implications for our concepts of the soil C and N cycle, soil structural stability and the sorptive properties. (Totsche *et al.* 2010).

The specific features of the novel nano-scale secondary ion mass spectrometry (nanoSIMS) technology, which allows the simultaneous analysis of up to seven ion species with high sensitivity and resolution, make it an unprecedented tool for the analysis of biogeochemical processes and properties of soils (Herrmann *et al.* 2007a, b). In the nanoSIMS, a beam of primary ions (Cs^+ or O^-) releases secondary ions from the sample. These secondary ions are collected and filtered by a magnetic mass analyser. With Cs^+ as primary ions, negatively charged ions, like *e.g.* $^{12}C^-$, $^{13}C^-$, $^{12}C^{14}N^-$, $^{12}C^{15}N^-$ and $^{28}Si^-$, are collected with a lateral resolution of 50 nm; by using O^- as primary ions beam, positively charged ions, like *e.g.* K^+ and Na^+, are detected with a lateral resolution of 150 nm. The mass resolution is so high that a differentiation between the mass of $^{13}C^{14}N$ (27.016 amu) and the mass of $^{12}C^{15}N$ (27.009 amu) is possible (*c.f.* Lechene *et al.* 2006). Consequently, the nanoSIMS enables us to explore the elemental and isotopic composition of soils at the submicron-scale.

Until now, the nanoSIMS technique has been applied mainly in the field of cosmochemistry, material science, biology, geology and mineralogy. We demonstrate the feasibility of this technique for soil science. After incubation of soil particle size fractions and soil aggregates with a ^{13}C and ^{15}N labelled amino acids

mixture, we traced the label at different time intervals on the single particles and in the aggregates. With this technique, we were able to show the isotopic enrichment of ^{13}C and ^{15}N on particle surfaces and the diffusion of the label among aggregate pores.

Methods

In this study, primary soil particles and soil aggregates were investigated with nanoSIMS with respect to the spatial distribution of freshly introduced organic matter (OM). The fine silt and clay soil particle size fraction (< 6.3 µm) of an Ah horizon from an Albic Luvisol from Bavaria (Southern Germany) was pre-incubated for 500 days at 20°C. Intact soil aggregates were taken from a Haplic Chernozem (Ap horizon, < 6.3 mm) from South of Kazan (Russia). Both soil materials were incubated with an amino acid mixture (approx. 99 atom% ^{13}C and ^{15}N) as readily bioavailable OM input and isotopic tracer. Samples were taken before, directly after the addition of the labelled amino acids and after 1, 2 and 6 days.

Sample preparation was carried out directly after sampling. The primary particles were deposited as a suspension on pieces of a silicon wafer and then dried in a desiccator. The soil aggregates were embedded in epoxy resin by stepwise combined drying and saturation of the soil aggregates with resin/acetone mixtures of different ratios. Then, the resin blocks were cut to obtain a transect through the aggregate and the obtained surface was polished with diamond paste. The samples were investigated with an optical microscope and a scanning electron microscope (SEM). The spatial distribution of the OM and the fate of ^{13}C and ^{15}N, which is expected to be influenced by diffusion, sorption and microbial activity, were studied with nanoSIMS. The presented nanoSIMS data were obtained at the nanoSIMS 50 unit of the Institut Curie in Paris, France.

Results

In the primary particle samples, particles were analysed by selection of “regions of interest” (ROI) and measurement of the elemental and isotopic composition of these sample areas. Figure 1 shows several particles high in carbon derived after 6 days of incubation. Ten different ROIs were selected and are marked with a red line. From these measurements, the isotopic ratios of these particles were calculated (Figure 2).

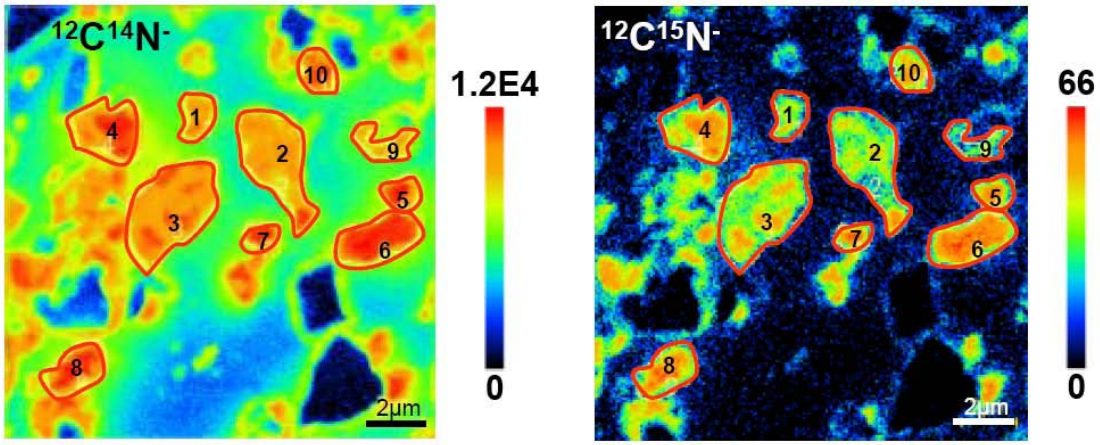

Figure 1. Spatial distribution of $^{12}C^{14}N^-$ and $^{12}C^{15}N^-$ at primary particles from the fine silt and clay fraction of the Ah horizon of the Albic Luvisol after 6 days of incubation. Investigated regions of interest are marked.

In Figure 2, it is clearly visible that a significant enrichment in both ^{13}C and $^{1}5N$ took place after addition of the labelled amino acid mixture. Over time, we detect a decrease in ^{13}C and ^{15}N, which is more pronounced for ^{15}N.

In the aggregates of the Haplic Chernozem, we detected that transport of the ^{13}C and ^{15}N labelled amino acids took place along a pore. Figure 3 shows a backscattered-electron image of the aggregate sampled after one day of incubation, in which the pore analysed by nanoSIMS is displayed. Moreover, the distribution of $^{12}C^{15}N^-$ in that particular pore is shown in the insert. The arrow indicates the direction of the line scan that was done; the small square indicates the width of it. The calculated isotopic ratios of the individual scan points along this transect demonstrate a decrease of ^{15}N towards the aggregate interior (Figure 4). Accordingly, we conclude that most likely diffusion of the labelled amino acids from the outside of the aggregate to its interior occurred along such pores.

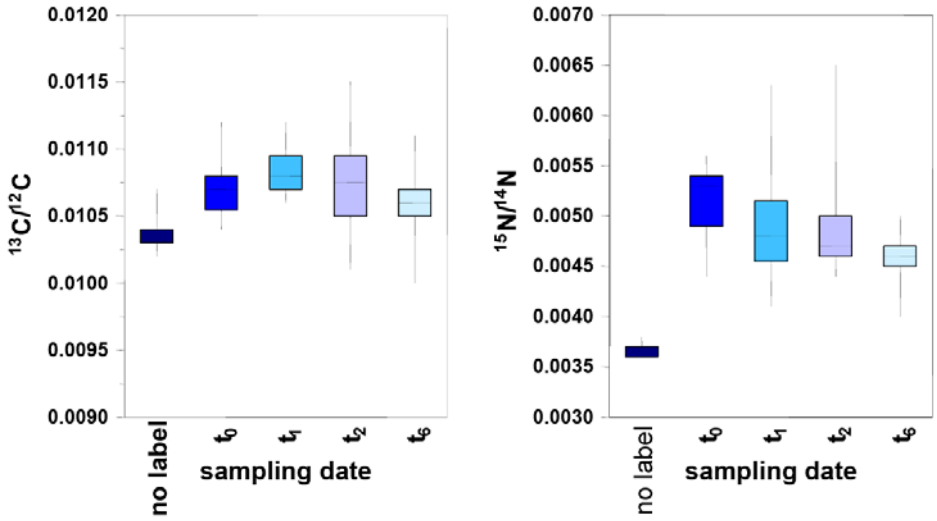

Figure 2. Development of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios calculated from measurements of selected regions of interest.

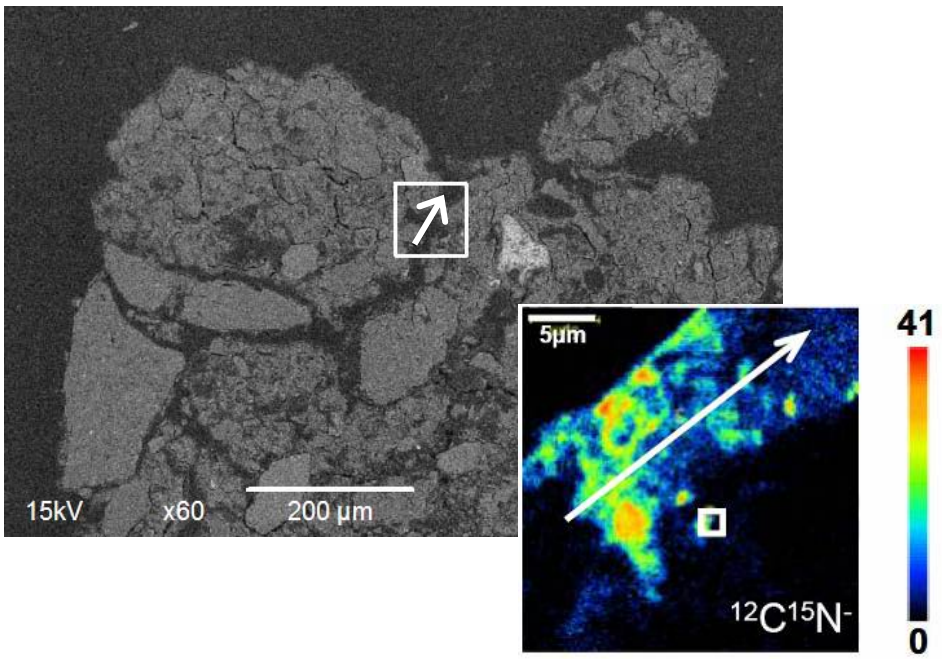

Figure 3. Backscattered-electron image of the aggregate of the Ap horizon of the Haplic Chernozem derived one day after incubation. The insert shows the spatial distribution of $^{12}C^{15}N^-$ along the investigated pore.

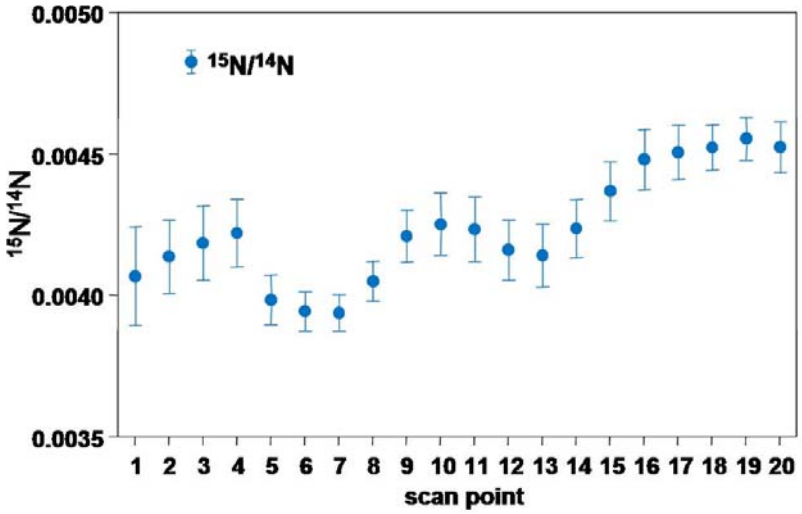

Figure 4. Variation of the $^{15}N/^{14}N$ ratio across the investigated pore of the aggregate.

Conclusions

The nanoSIMS technology enables us to explore the elemental and isotopic composition of soils at the submicron-scale. We developed sample preparation procedures to obtain samples that meet the enhanced requirements of the nanoSIMS for investigation of both primary soil particles as well as soil aggregates. An incubation experiment with primary particles of an Albic Luvisol and with soil aggregates of a Haplic Chernozem was conducted with a ^{13}C and ^{15}N labelled amino acid mixture. NanoSIMS analyses reveal that after label addition, primary soil particles high in carbon show enrichment in both ^{13}C and ^{15}N which decreases with time. In soil aggregates, nanoSIMS analyses illustrate the transport, which is most likely by diffusion, of the labelled amino acids from the outside to the interior of the aggregates along their pores. From these first results, it can be concluded that the nanoSIMS technology will boost our ability to locate the association of elements/isotopes in soil structural components at the submicron-scale and will thus allow a major step forward in the understanding of biogeochemical processes and properties of soil.

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