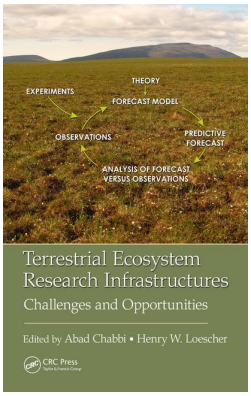

Terrestrial Ecosystem Research Infrastructures Challenges and Opportunities

Abad Chabbi, Henry W. Loescher

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Carsten W. Mueller, Laurent Remusat, Cornelia Rumpel
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Characterization of Biogeochemical Processes at the Microscale: Concepts and Applications of NanoSIMS

Carsten W. Mueller, Laurent Remusat, and Cornelia Rumpel

CONTENTS

Abstract

Nanoscale secondary ion mass spectrometry (NanoSIMS) is a state-of-the-art analytical technique allowing for the visualization of the distribution of up to seven isotopes in environmental samples at high lateral resolution. The use of this technique combined with stable isotope labeling and other microscale methods will advance our understanding of biochemical cycles. Particularly, NanoSIMS has a high potential to generate new knowledge concerning functioning of microbial communities and biogeochemical interfaces because it allows for studying biogeochemical processes at the relevant scales, where they occur. In this chapter, we present this technique and its limitations and applications in recent biogeochemical research. Moreover, we show through some examples of significant advances achieved by the use of NanoSIMS how this may lead to changing paradigms in environmental sciences.

8.1 Introduction

Large-scale biogeochemical processes (e.g., C cycling) are driven by micro- to nanoscale interactions among microorganisms, mineral constituents, and natural organic matter (Schmidt et al., 2011). At the same time, small-scale processes often show faster reaction rates in isolated zones ("hot spots"), in comparison to adjacent areas. Such hot spots occur at terrestrial–aquatic interfaces (McClain et al., 2003) or, in terms of microbial activity, at specific biophysical microenvironments, for example, the rhizosphere (Kuzyakov and Blagodatskaya, 2015; Young and Crawford, 2004). In soils and sediments, there are many biotic hot spots located within the mineral matrix, including those generated by bioturbation and microbial or plant activity (Hinsinger et al., 2009; Young et al., 1998). Recently, Falconer et al. (2015) simulated the effect of structural heterogeneity on microbial respiration. Using mathematical simulations, the authors showed that larger particulate organic matter was metabolically easier to decompose than smaller, unevenly distributed particulate organic matter, due to a so-called insulation of the fungal colony within the soil structure (Falconer et al., 2015). This and other works imply the great need for more refined data, obtained at the relevant scale, where microbial transformations take place. But most biogeochemical research is conducted using large bulk (>1 g) samples, which are usually strongly altered prior to analysis (e.g., grinding, solvent extraction). Furthermore, the investigation of intact soil/sediment structures and microbial ecosystems at the scale of so-called microhabitats, for example, hot spots of increased organic matter turnover and higher microbial activity, is usually prevented by the destruction of intact soil/sediment structures by most bulk sampling techniques. A lack in progress concerning the deeper understanding of organic carbon dynamics is mainly due to the restricted answers obtained by macroscopic measurements (Baveye, 2015). Hence, to gain a deeper understanding of the elemental fluxes (C, N, P, and S) among plants, microorganisms, and minerals in complex environments, it is necessary to study these elemental constituents at the spatial scale relevant for biogeochemical processes. Characterizing the spatial heterogeneity at soil microenvironments with respect to microbial ecology and upscaling of their importance for global cycles was named as one of the major challenges in ecological science (Baveye, 2015). It was further suggested that classical concepts in biogeochemical research would substantially evolve in the future by adapting recent approaches used in modern microbiology (Baveye, 2015). Consequently, the preparation of samples should clearly aim to maintain the structural integrity that keeps microorganisms and microstructures intact and avoids their dislocation in order to be able to study biogeochemical processes in intact microenvironments. Thus, to gain a real comprehensive understanding of how biogeochemical processes are determined by organic matter composition, microbial activity, and soil/sediment

physical structure, it is crucial to integrate biogeochemistry, microbiology, and physics (Baveye and Laba, 2015; Dijkstra et al., 2013). Great technical developments over the last years now provide access to a number of analytical instruments (e.g., x-ray tomography, x-ray spectromicroscopy) to study microenvironments at previously unresolved scales (Baveye, 2015). Especially, the nanoscale secondary ion mass spectrometry (NanoSIMS), capable of the visualization of stable isotopes up to a lateral resolution of 50 nm, has revolutionized our view on microscale biogeochemical processes. The use of NanoSIMS to explore soil processes in particular is the most recent application for this rather new SIMS technique, which was previously mainly used in cosmochemistry, geology, microbiology, and life science (Hoppe et al., 2013; Moore et al., 2011; Musat et al., 2009). By using NanoSIMS in concert with other imaging techniques (e.g., scanning transmission x-ray microscopy [STXM], scanning electron microscopy [SEM]), classic analytical analyses (isotopic and elemental analysis) and biochemical methods (NMR spectroscopy, GC-MS), it is now possible to exhibit a more complete picture of environmental processes at the microscale, which already yielded results allowing to challenge existing paradigms (Keiluweit et al., 2012; Liu et al., 2013; Moore et al., 2011; Mueller et al., 2013; Remusat et al., 2012; Vogel et al., 2014).

8.2 SIMS Principle, Technical Requirements, and Limitations

NanoSIMS is a destructive spectromicroscopic technique in which a primary ion beam is sputtering the surface of a solid sample yielding detectable secondary ions. The acceleration of primary ions (about 16 keV) results in a depth resolution of approximately 10 nm; hence, NanoSIMS can be considered as a technique to assess surface chemistry. It allows for the study of a much smaller sample volume in comparison to other microscopic techniques, like SEM, which integrate often over several μm in depth due to larger-scale interaction between the incident particle and the sample atoms and molecules. Together with the ability to visualize the distribution of isotopes at a high lateral resolution, NanoSIMS is an unprecedented tool for microscale studies in biogeochemistry (Hoppe et al., 2013). The improved primary ion optics and secondary ion transmission at high mass resolving power, in contrast to large geometry SIMS (e.g., Cameca 1280), enable the Cameca NanoSIMS to image isotopes with high sensitivity (lower than ppm levels) at the submicron scale. Using cesium as primary ion (Cs^+), NanoSIMS analysis is able to reach a theoretical spatial resolution of ~50 nm using very low primary currents (less than 1 pA). However, in order to still yield a statistically sufficient amount of secondary ions from natural samples, the primary ion beam is set to a few pA, resulting in ~100–150 nm lateral resolution. This is, for example,

sufficient to differentiate between specific sulfur-rich domains on extracellular polysaccharides–covered goethite needles with a size of a few hundred nanometers (Liu et al., 2013). This study indicates nicely the elemental imaging capabilities of organomineral sample material at the upper spatial resolution boundary. Due to the coaxial setup of the NanoSIMS, allowing for the sharp focusing of the primary ion beam and thus the high lateral resolution, primary and secondary ions have to be of different polarity. NanoSIMS is theoretically capable of detecting masses from 1 to 400 amu. Up to seven secondary ions can be simultaneously detected in the multicollection chamber, with some limitations imposed by its design: there is a maximum factor of 21 between the lightest and heaviest ions that can be collected simultaneously. This enables investigating a wide range of biogeochemical interesting isotopic systems by the detection of species released from the main constituents of organic matter (Cs^+ as primary ion: $^{12}C^-$, $^{13}C^-$, $^{12}C^{14}N^-$, $^{12}C^{15}N^-$, $^{31}P^-$, $^{32}S^-$) or minerals (Cs^+ as primary ion: $^{28}Si^-$, $^{27}Al^{16}O^-$, $^{56}Fe^{16}O^-$). Nitrogen is detected as cyanide (CN^-, molecular ion) due to the very low ionization of the nitrogen atom under the Cs^+ (McMahon et al., 2006). Aluminum and iron (and elements like Ca, Na, or metals being more akin for positive ionizing) can be detected as molecular ion together with oxygen when using the Cs^+ primary ion beam. It must be noted that for studying the distribution of metals, for instance, aluminum or iron, an O^- source, producing cations like $^{27}Al^+$ and $^{56}Fe^+$, is more suitable than a Cs^+ source. However, the conventional O^- source has low brightness hampering the lateral resolution at approximately 300 nm. Very recent developments on a new design of the oxygen source have led to significant improvements of the lateral resolution (down to 50 nm), unleashing great opportunities for the study of the distribution of Mg, Ca, or K. Nevertheless, the O^- source is not suitable to investigate organic matter as C and N ionize poorly with O^- primary ions.

Although analyses of natural abundance ^{13}C and ^{15}N in organic matter are widely used in biogeochemistry, very few NanoSIMS studies investigate the distribution of isotopic ratios from untreated natural samples because the NanoSIMS precision for isotope ratios is limited to ~1‰ in imaging analytical conditions (Slodzian, 2004). To obtain the high precision (<0.5‰) required to disentangle biogeochemical processes, large amounts of sample material have to be retrieved from the sample surface (nanograms), which requires large primary spots of about ~10 μm; this is achieved by large SIMS instruments like the IMS 1280. But such spot size is beyond the relevant scale for most biogeochemical processes (e.g., microorganisms, primary minerals). Consequently, the use of isotopic tracers to study microscale processes can be seen as the most promising way to obtain relevant results with NanoSIMS in biogeochemistry as described in this chapter. Adding constituents (for instance, organic compounds) enriched in specific stable isotopes results in contrast enhancement in isotope images because the range of isotopic composition in the locations of the label is significantly different from those of unaffected areas.

Aside from the technical constraints of the instrument itself, collection of meaningful data to elucidate environmental processes depends on the selection and preparation of suitable samples. Identifying and isolating a nanomaterial being represented at a larger scale and addressing its spatial heterogeneity may be crucial for the interpretation of results. Moreover, instrumental constraints require that the samples be vacuum stable, as flat as possible and conductive (Mueller et al., 2013). The fundamental requirement is the stability under high vacuum (up to 10^{-11} mbar), which excludes samples that either strongly degas (e.g., thick resin-embedded sections) or have higher amounts of residual water (e.g., fresh biological tissues). Due to the high sensitivity of the NanoSIMS, another key challenge is how to deal with the occurrence of contaminations, which is an important issue especially for the classical sample embedding using epoxy or polyester resins. Such resins contain C and N, which can dilute isotopic signals from the sample itself and hamper the spatial differentiation between resin and C and CN in the organic matter of the sample. Systematic errors can be avoided by using adequate embedding materials, such as the resin Araldite 502 (Figure 8.1; Herrmann et al., 2007) or sulfur (Lehmann et al., 2005). Other studies used special computer techniques to differentiate resin-derived elements and those from the actual samples (Rennert et al., 2014). A possible alternative is avoiding embedding agents during sample preparation, for example, by applying cryofixation and cryosectioning (Mueller et al., 2013) or an erbium oxide marker layer to avoid any mixing with the organic matter of the soil sample (Hoeschen et al., 2015).

Systematic errors through resin embedding and sectioning can also be circumvented by using sedimentation and deposition of fine grain samples (e.g., clay-sized fractions) on Si wafers (Vogel et al., 2014) or gold foil (Hatton et al., 2015). As Si wafers are very flat and conductive, they are optimal as sample substrate for minerals but also thin sections from biological tissues. Nevertheless, the deposition of particles on a flat carrier material does not prevent the occurrence of topography issues. The use of the technique reported by Hoeschen et al. (2015) to produce flat sample sections can yield promising results at substantially reduced topography. Especially in microbiology, gold-coated polycarbonate filters are used as sample substrate for microbial cells (Miot et al., 2015; Musat et al., 2009). These allow for the combination of molecular techniques with subsequent visualization using microscopic techniques in combination with NanoSIMS (Musat et al., 2009).

Other systematic errors may include the representativeness of the sample to be analyzed as well as the reproducibility of the obtained data. Especially with respect to large-scale phenomena, it will be crucial to put more efforts on upscaling approaches. This is a major issue, when NanoSIMS analyses are carried out on complex material like soil. In this case, particles of a specific fraction are selected, allowing to address specific questions, for instance, with regard to soil organic matter stabilization. For this kind of analyses, sample choice and preparation are extremely important in order to avoid errors.

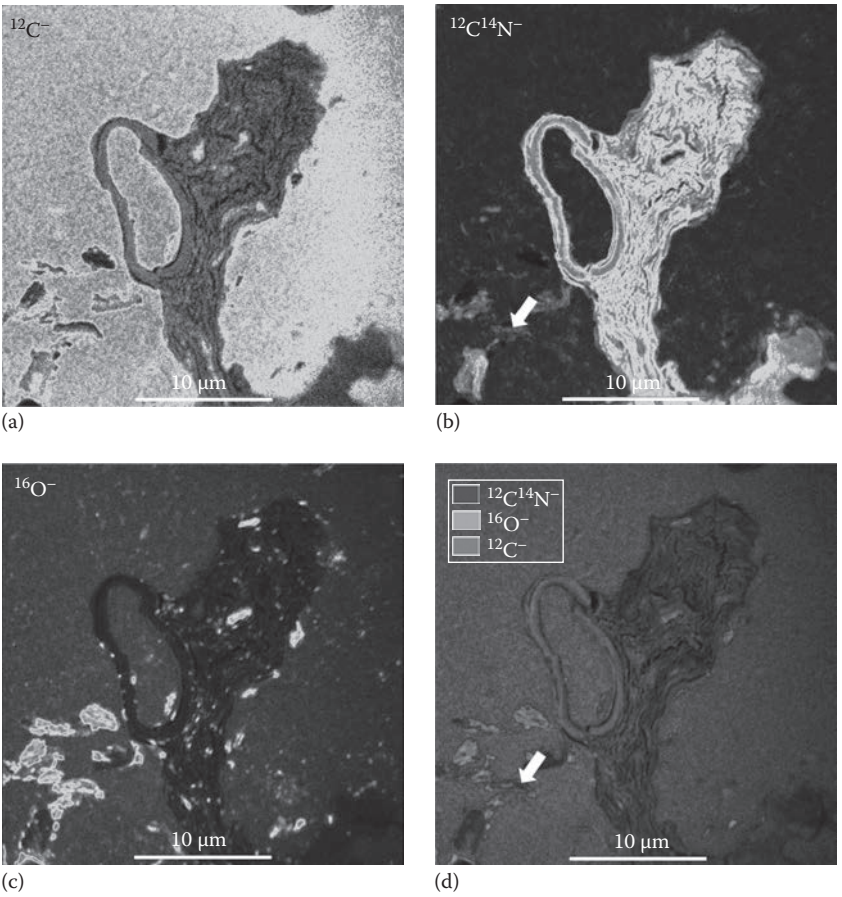

FIGURE 8.1
(See color insert.) NanoSIMS images of the distribution of $^{12}C^-$ (a), $^{12}C^{14}N^-$ (b), $^{16}O^-$ (c), and a composite image of the three secondary ions (d) derived from a resin-embedded section of a particulate organic matter–rich permafrost soil structure (Typic Aquiturbel, Barrow, Alaska). The distinctive different distributions between secondary ion counts of $^{12}C^-$ and $^{12}C^{14}N^-$ (d) indicate the good spatial differentiation between the used epoxy resin (a, Araldite 502) and the soil organic matter (b). The $^{16}O^-$ shows the distribution of mineral particles; the arrow indicates mineral-associated organic matter. (From Mueller, C.W., TU Munich, Germany, unpublished data.)

This concerns not only the specific sample preparation as outlined earlier but also the choice of the actual sample and the region of interest to be analyzed. Users of the technique should be clear about the mechanisms to be analyzed, which will determine sample choice and preparation. For complex systems like soils or sediments, the sample preparation protocol has to be adjusted almost for every new set of samples, trying to minimize artifacts and contaminations while aiming for preferably flat conductive samples. The analysis of microbial cells in soils is usually hampered due to their dispersal in a large

background of particles. Recently, an efficient sample preparation technique for the extraction of single microbial cells from soils for NanoSIMS analysis was introduced (Eichorst et al., 2015).

In order to upscale NanoSIMS results, it will be tremendously important to combine these nano- to microscale explorations with techniques suitable for use at larger scales (Baveye, 2015). Techniques like laser ablation ICP-MS but also imaging techniques like VIS-NIR imaging might represent constructive tools for upscaling. Further development in digital image analyses, as recently demonstrated by Rennert et al. (2014), and geostatistical approaches (Mueller et al., 2012, 2013) could constitute key techniques to set NanoSIMS results in a larger perspective. These techniques allow for quantitative exploitation of the visual distribution of the isotopes detected by NanoSIMS. The colocation of labeled and unlabeled components at the microscale may thus be evaluated statistically and compared to their distribution at larger scales. This is a first step to upscale the NanoSIMS results. Another approach may include the integration and testing of concepts derived from microscale observations by modeling to verify if they improve the prediction of large-scale phenomena.

8.3 Application in Biogeochemistry

The NanoSIMS offers the capabilities to image stable isotopic ratios at the scale, where biogeochemical processes occur in a broad range of samples ranging from soils to sediments and living organisms. However, most of the natural variance in stable isotopic quantities occurs within a few per mil. As stated earlier, the current instrument limitations prevent for measuring sub-per mil precision isotopic ratios in micron-scale objects using the imaging mode of the NanoSIMS instrument. There is nevertheless a huge field of applications when considering isotopic labeling experiments, for example, element uptake, sorption, and microbial use. Isotopic labeling is a powerful tool to shed light on biogeochemical processes, as it permits to track a specific element of interest (C, N, etc.) without modification of its concentration or induction of radioactivity. Overall, when performing an isotopic labeling experiment, a labeled substrate is introduced and behaves in the same way as an unlabeled substrate. Therefore, all biogeochemical processes will happen in the same way but they are observable via the isotopic label. In pulse-chase experiments, for example, the label is brought into the system at the beginning of the experiment, during short-term exposure of the system to isotopically labeled compounds (isotopic pulse). Thereafter, the transfer of the labeled elements through different compartments of the system can be followed: transformations of the initial compounds and their chemical pathways can be elucidated. By sampling

at different times after the isotopic pulse, it becomes possible to assess timescales for the investigated processes, allowing for the analyses of reaction rates of biogeochemical processes. NanoSIMS imaging is the ideal tool to reveal the location of this isotopic label at the microscale, allowing for visualization of the labeled compounds in compartments (microaggregates, plant or microbial cells) of the studied biogeochemical system. Studies with labeled material are useful to elucidate microbial functioning under varying environmental conditions. Isotopic labeling of the microbes itself may allow for studying their ecology, networks, and their interaction with organic and inorganic surfaces.

Many studies in the field of environmental science and biogeoscience have reported insights gained from NanoSIMS imaging of samples labeled with stable isotopes. Such experiments may be performed in order to visualize the uptake of ^{13}C- or ^{15}N-labeled nutrients (Musat et al., 2009), like acetate, or a chemical element relevant for the system, like Mg or Ca in organisms precipitating carbonate minerals, bringing constraints on transfers and cycling in biogeochemical systems. In soil science, stable isotopic labeling was employed to reveal N uptake in the rhizosphere (Clode et al., 2009). Double-labeling experiments have revealed that N and S cycling rates differ in their fate during organic matter digestion by earthworms (Gicquel et al., 2013) or that C and N cycles are rapidly decoupled by microbial activity in top soils (Hatton et al., 2015). Experiments with labeled organic matter can provide compelling advances on the processes that govern organomineral associations (Keiluweit et al., 2012; Vogel et al., 2014). In marine environment, Lechene et al. (2007) have followed the transfer of N inside animal cells subsequent to N_2 fixation by endosymbiotic bacteria. Organic matter transfers can therefore be evaluated thanks to stable isotopic labeling and imaging of the label down to processes occurring within single cells.

Recent developments on the combination of NanoSIMS with stable isotopic labeling have been fruitful in the studies of interactions between life and minerals. Living systems can be subjected to biomineralization: cells are then associated with the precipitation of various kinds of phosphate, carbonates, or oxides. In some cases, this is achieved to get a benefit, as for coral biomineralization. Brahmi et al. (2012) developed a methodology using ^{86}Sr pulse-chase labeling experiments to determine the extension rate of specific components of the coral skeleton. The combination of ^{86}Sr, ^{44}Ca, or ^{26}Mg isotopic labeling with NanoSIMS imaging sheds new light on the processes governing the growth of calcareous structures formed by organisms (Domart-Coulon et al., 2014; Gorzelak et al., 2014; Nehrke et al., 2013). Corals are very sensitive to concentration variations in their environment, but they do not notice isotope variations. In these studies, the addition of the stable isotope label did not significantly alter the concentrations in the growing media. Coral organisms were then grown in conditions very close to the natural environment thus avoiding disturbing their physiological

development. Stable isotopic labeling is also a valuable tool to assess symbiotic relations in reef corals. Pernice et al. (2012) studied the ammonium assimilation in a similar association for the *Acropora aspera* reef coral, using ^{15}N-rich ammonium. Kopp et al. (2015) investigated inorganic C and N uptake, as bicarbonate and nitrate, respectively, in the symbiotic reef coral *Pocillopora damicornis*. In both cases, they were able to show that the dinoflagellate symbionts are responsible for the inorganic C and N uptake. In addition, they could prove that symbionts are transferring C and N as organic compounds to the reef coral. In other cases, biomineralization can be induced as a reaction to the environment composition due to cell activity or/and super saturation of phosphates or carbonates. This process, however, has often a deleterious consequence on cells, by preventing cell division or transmembrane transport. As such, Miot et al. (2015) investigated how *Acidovorax* sp. strain BoFeN1, a bacteria known to reduce nitrate and oxidize Fe(II), can overcome death due to biomineralization. The assimilation of ^{13}C-labeled acetate was used as a marker for viability (Figure 8.2). It appears from this experiment that biomineralization leads to cell death and that a few individuals remain mineral-free, due to some phenotypic heterogeneity, and hence alive. These individuals will divide once the conditions are favorable, ensuring the community viability.

As NanoSIMS cannot characterize molecular properties, a significant step forward may be achieved when NanoSIMS imaging is combined with other imaging techniques, or any other means to identify the type of organic compounds or microorganisms that are sampled by the ion beam. However, techniques used should work at a comparably meaningful scale(s). Transmission electron microscopy (TEM) is addressing similar scales and can be used to provide additional structural identification of cells or tissues (Lechene et al., 2007; Vidal et al., 2016). Hybridization techniques, like fluorescence in situ hybridization (FISH), HISH, or Card-FISH, can identify microbial species at a single-cell level by the tracking of specific DNA sequences (Dekas et al., 2009; Musat et al., 2008). The molecular signature of organic material can be investigated at the micron scale by STXM or Raman microspectroscopy in order to distinguish plant- or bacteria-derived organic matter (Keiluweit et al., 2012; Liu et al., 2013; Remusat et al., 2012). By combining the NanoSIMS with such techniques, an isotopic composition can be attributed to a type of cell or organic matter, shedding lights on the origin of the component trapping the stable isotope label (e.g., Figure 8.3). Using TEM, microorganisms are identified without ambiguity, and it is possible to distinguish bacteria from fungi or plant residues. Such identifications may be challenging with NanoSIMS images, alone. With joint comparison of the sample using both NanoSIMS and TEM approaches, it becomes possible to specifically ascribe an isotopic ratio to a type of microorganism. In a study by Vidal et al. (2016), a labeled litter was amended to a soil in the presence of earthworms. Twenty-four weeks after the beginning of the experiment, casts produced by earthworms were sampled and mounted for both TEM and NanoSIMS imaging. Results

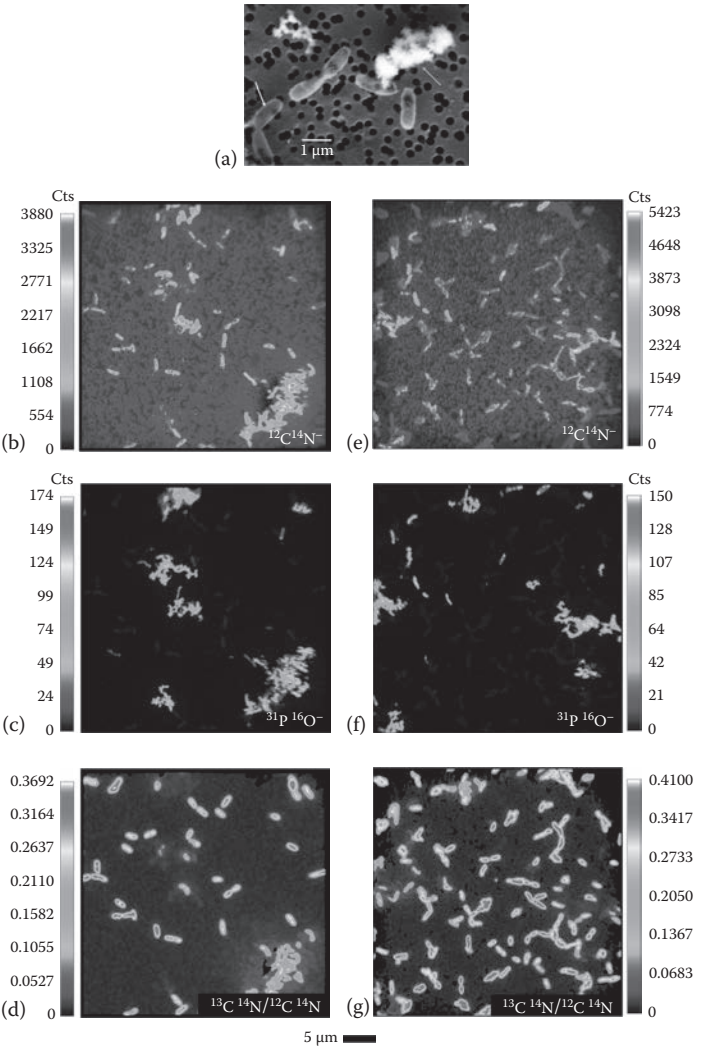

FIGURE 8.2
(**See color insert.**) *Acidovorax* sp. strain BoFeN1 is inducing Fe mineralization (here Fe-phosphate, image a, orange arrow) as a consequence of Fe oxidation in the presence of large amount of dissolved phosphate in the environment. A cell culture was exposed to the mineralization media for 4 days before being transferred to a media with ^{13}C-labeled acetate and no dissolved phosphate. Cells were then harvested after 1 day (NanoSIMS images b–d) or 4 days (e–g) to visualize the evolution of the bacterial colony. CN images (b, e) show the cells location, PO images (c, f) the precipitated minerals. The ^{13}C-enrichment (d, g) reveals the assimilation of acetate by living cells; this is a marker of active bacteria (e.g., living cells). Cells showing significant mineralization are free of ^{13}C-enrichment, showing that mineralization induces cell death. (Modified from Miot, J. et al., *Front. Microbiol.*, 6, 2015.)

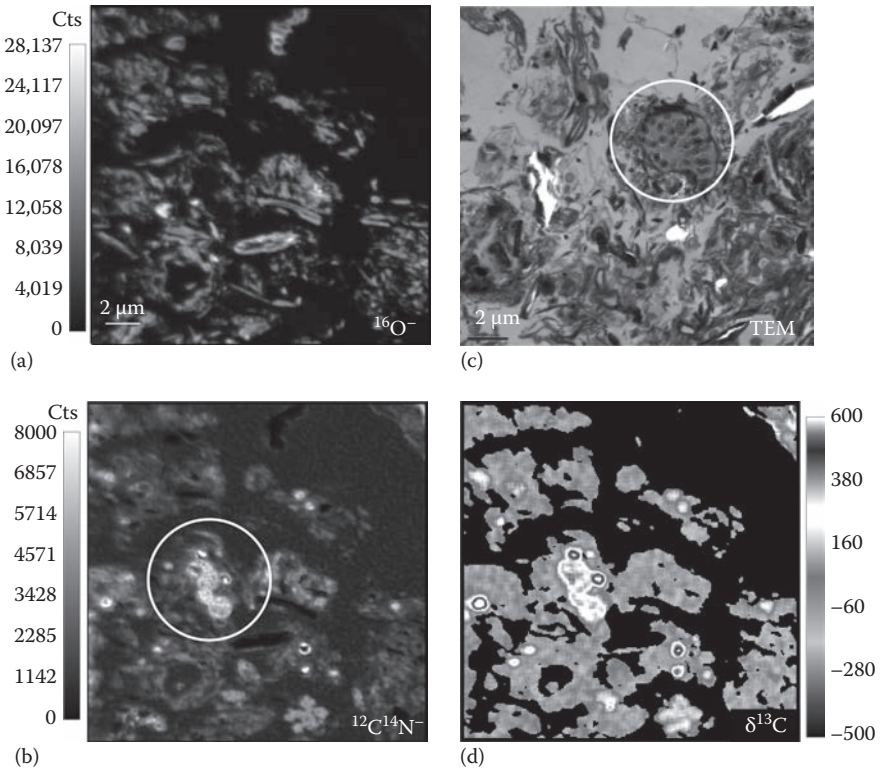

FIGURE 8.3
(**See color insert.**) NanoSIMS and TEM (top right) images of an earthworm cast recovered after a 24-week labeling experiment. ^{16}O image (a) is showing minerals, the $^{12}C^{14}N$ image (b) the organic matter. Thanks to the TEM image (c), we can identify the typical morphology for bacterial colonies and we observe that bacteria have trapped C from the labeled litter (shown by the $\delta^{13}C$ image (d)). (Modified from Vidal, A. et al., *Soil Biol. Biochem.*, 93, 8, 2016.)

showed that bacteria living within the cast had fixed C from the litter, demonstrating that earthworms incorporated fresh organic matter from the litter and facilitate the delivery of the substrate to decomposer microorganisms.

8.4 Main Achievements in Soil Science Thanks to NanoSIMS Analyses

8.4.1 Characterization of Microbial Communities

Biogeochemical cycles are intimately linked with microbial ecology and microbial ecophysiology. In microbial ecology, great progress has been made

in the characterization of microbial community dynamics with the development of 16S and 18S rRNA sequencing and environmental genomics (Tringe et al., 2005). These techniques have revolutionized microbial ecology by identifying a huge genetic diversity of microorganisms in environmental samples, mainly bacteria, associated with functional potential. However, these approaches were successful in determining the presence of microorganisms but not in discerning their function or whether they were active or not. Thus, making functional relationships with other environmental parameters (soil type, water availability, season, substrate, temperature, etc.) was hardly possible (Wagner, 2009). This relationship may now be studied at the single-cell level and therefore at the scale at which microbial processes occur, thanks to the high lateral resolution of NanoSIMS (Li et al., 2008). NanoSIMS analyses, especially when combined with stable isotope labeling, provide deep insight into the functioning of single cells (Musat et al., 2012; Wagner, 2009). With this analytical breakthrough, characterization of microbial processes driving global biogeochemical cycles has become possible and thus changed some of the existing paradigms (Behrens et al., 2008, 2012; Musat et al., 2012; Wagner, 2009). For example, it has been shown that phylogenetics are in many cases not related to function and that therefore the well-established assumption of linkages between phylogenetic and functional prediction need to be revised even for closely related taxonomic groups (Mayali et al., 2012). This illustrates that NanoSIMS may be able to advance our understanding about the relationship between taxonomy/systematics on the one hand and function/activity on the other. Other studies successfully investigated important ecophysiological processes at the single cell level, such as uptake of elements (Behrens et al., 2008; Popa et al., 2007; Zimmermann et al., 2015). They indicate a large heterogeneity of the temporal and spatial nature of element uptake within and among individual microbial cells due to specific metabolic rates of single cells of the same species (Behrens et al., 2008; Popa et al., 2007; Zimmermann et al., 2015). Moreover, NanoSIMS analyses also revealed that species present in minor abundances may contribute greatly to biogeochemical processes (Musat et al., 2008).

Progress was also made at the process level through the visualization of carbon and nitrogen fluxes within the atmosphere–soil–plant continuum at the single-cell level (Clode et al., 2009). NanoSIMS now allows distinguishing elemental uptake at biological interfaces such as root and mycorrhiza hyphae (Nuccio et al., 2013). Thus, new experiments were set up to analyze in detail plant–microbial interactions, including mycorrhizal fungi. The results allowed for a better understanding of the quantitative importance of organic and inorganic N forms for plant nutrition. It was confirmed that NH_4^+ is the major contributor to plant N nutrition, whereas organic N forms are mainly used by C-limited soil microorganisms (Jones et al., 2013). NanoSIMS results further indicated that *arbuscular mycorrhizae* could control litter decay by influencing the litter–decaying microbial communities and through N export (Nuccio et al., 2013). *Arbuscular mycorrhizae* is also an effective distributor of

recently assimilated plant-derived C to soil microorganisms (Kaiser et al., 2015). By this process, plants may be able to actively control the microbial communities involved in the decomposition and hence nutrient release from soil organic matter.

8.4.2 Biogeochemical Functioning of Soil Primary Particles and Microaggregates

Soil comprises a complex hierarchical organization characterized by a heterogeneous spatial arrangement of mineral particles, organic matter, and biota. This structural organization as well as its biogeochemical functioning control water, gas, and energy flow in soils. As already mentioned earlier, the understanding of the biogeochemical functioning of soils has long been hampered by the analyses of bulk soil samples, ignoring the intrinsic complexity of soil, that is, the 3D arrangement of minerals, organic matter, soil (micro-) organisms, water, and air. Many microbial processes determining important soil properties and ecosystem services such as carbon storage and nutrient release occur at distinctive hot spots at micro- to nanoscale biogeochemical interfaces (McClain et al., 2003; Totsche et al., 2010). Scientific advances were made with NanoSIMS using isotopic labeling to visualize the incorporation of organic C, N, and S into the soil matrix. In particular, the heterogenic distribution and composition of organic matter (OM) has been evidenced at soil mineral surfaces. With this technique, major advances concerning the stabilization processes of OM have been made. The paradigm of C storage potential of soils depending on the availability of mineral surface area free of OM has been questioned due to recent results with NanoSIMS (Vogel et al., 2014). By using short-term incubation and isotopic labeling, it has been shown that new OM is sorbed on already preexisting clusters, indicating that only a limited proportion of the total mineral surface area may be available for C sequestration (Hatton et al., 2015; Vogel et al., 2014). These results could strongly impact the way that C storage potential of soils is determined.

NanoSIMS has led to great progress in the understanding of the origin of OM stabilized at mineral surfaces. Soil incubation studies using labeled organic material, supported the preferential binding of microbial-derived OM at mineral surfaces by combining NanoSIMS isotopic analyses with compositional information derived from STXM measurements (Keiluweit et al., 2010; Remusat et al., 2012). The increased binding of microbial-derived OM known from bulk analyses (Kleber et al., 2007) was also supported by the approach of Hatton et al. (2012), who used the elemental composition (^{12}C, $^{12}C^{14}N$) of OM at mineral surfaces measured by NanoSIMS in comparison to bulk C/N ratios from classical approaches and showed that microbial-processed material is stabilized preferentially (Hatton et al., 2015). In situ studies, using long-term field experiments in combination with NanoSIMS, showed that microbial recycling of OM is spatially heterogeneous (Remusat et al., 2012)

and depending on soil depth (Rumpel et al., 2015). It is assumed that this may be more intense in microbial hot pots and in subsoil horizons as compared to topsoil (Rumpel et al., 2015). Moreover, different OM compounds may be affected by this process in different depths of the soil profile (Rumpel et al., 2015). Decoupling of C and N during the stabilization process, as indicated for mineralization experiments from bulk analyses (Bimueller et al., 2014), has been noted at the microscale by several studies (Hatton et al., 2015; Rumpel et al., 2015). These findings show that phenomena existing in bulk soil also occur at the microscale. The application of these more detailed analyses to generate process information led to understanding of the coupling of biotic and abiotic controls on biogeochemical cycling. For example, very recently the paradigm of long-term stability of mineral-associated OM was challenged using a combination of NanoSIMS and STXM (Keiluweit et al., 2015). It seems that mineral-associated OM may be liberated through the addition of labile substrates such as those present in root exudates. Other studies addressed biogeochemical processes at biological interfaces created by earthworm activity. While earthworms were found to be of great importance for physical soil properties, such as aeration and water infiltration, only little is known about their impact on small-scale biogeochemical cycling of elements between plant, microorganisms, and soil. As stated earlier, NanoSIMS analyses showed the tight interaction between the OM uptake of the earthworm and the resulting enrichment of associated microorganisms in the mineral-dominated earthworm casts (Figure 8.3) (Gicquel et al., 2013; Vidal et al., 2016). These findings show that great progress in the understanding of the coupling of biotic and abiotic controls on biogeochemical cycling has already been made, and is to be expected by application of the NanoSIMS technique to environmental samples.

8.5 Summary and Outlook

Due to the growing number of facilities and easier access to spectroscopic and spectrometric imaging techniques, we see an advent of the study of biogeochemical processes at the microscale. Some of these studies led to a change of paradigms concerning our understanding of microbial ecology and elemental cycling in marine as well as terrestrial environments. Even if these proof-of-concept studies show that process information concerning microbial functioning as well as elemental cycles at biogeochemical interfaces may be obtained at the relevant scales, many issues concerning the use of NanoSIMS in environmental studies remain unresolved. There are still some technical and conceptual developments required to enhance the scientific outcome of microscale explorations. As stated earlier, the quantification of soil/sediment chemical, physical, and biological complexity is an urgent

need to underpin large-scale elemental cycles. Nanoscale information on the interactions between the different living and nonliving parts of ecosystems are crucial to understand the processes behind natural phenomena. We need the combination of several classical and modern spectromicroscopic tools in order to address all these different aspects. NanoSIMS is one of these tools, which can provide additional information on compound dynamics thanks to the measure of stable isotopes. Upscaling of the information obtained at microscale is a critical issue, even if there is some evidence that processes occurring at the small scale may also be seen at larger scales. The understanding of environmental systems, as a whole, will only benefit if microscale measurements are incorporated in models considering a multitude of factors of the "3D porous medium." There is a need for new scientific concepts, experimental designs, and analytical protocols to enable the future exploration of intact soil and sediment samples at the process scale. The available techniques at different scales ranging, for example, from x-ray computed micro-tomography (μ-CT) and x-ray photoelectron spectroscopy to electron microscopy (SEM and TEM) and synchrotron-based analyses (STXM) will, together with spatially high-resolved isotopic information (NanoSIMS), set the base for a new generation of scientific concepts of biogeochemical processes and enable the development of more accurate multifunctional process models. In order to fully understand the complexity of biogeochemical microenvironments and their structures, traditional macroscopic and new microscopic techniques have to be used in scientific sound concert. In order to advance our understanding of the functioning of natural ecosystems, NanoSIMS and other microscale techniques need to be part of ecosystem research infrastructures, allowing for meaningful experiments to generate process information. This new information may then be tested in ecosystem models addressing large-scale processes with measured data obtained from monitoring facilities within ecosystem research infrastructure networks.

Glossary

Sputtering: Physical process resulting from the interaction of incident primary ions with the surface of the sample. Primary ions impact the surface and induce a cascade of collisions between the atoms of the sample. This process leads to the formation of secondary ions.

TEM (transmission electron microscopy): Transmission microscopy technique using a focused electron beam for nanometer-scale structural characterization and elemental analyses.

STXM (scanning transmission x-ray microscopy): Synchrotron-based x-ray imaging technique used to characterize, for instance, organic matter at the nanometer scale (spatial resolution 50 nm). It provides spatially resolved XANES (x-ray absorption near-edge structure)

spectra. A XANES spectrum reports the absorption of x-rays of specific energies for electronic transitions and provides information about the chemical environment of a desired chemical element (e.g., functional groups in the case of C-XANES).

FISH (fluorescence in situ hybridization): Cytogenetic technique with fluorescence probes to mark specific DNA sequences, which are detected by fluorescence microscopy. CARD-FISH is its combination with catalyzed reporter deposition for signal amplification. In some experiments, the fluorescent tag can be replaced by a halogen-based tag that is easily detected by NanoSIMS (FISH-SIMS).

Raman microspectroscopy: Vibrational technique used to characterize the structure of organic matter and minerals. A monochromatic laser beam interacts with the molecular vibrations in the sample and the energy of the laser photons is shifted. This shift provides the vibrational modes in the sample, hence hints on the structural properties. Raman spectroscopy is complimentary to infrared spectroscopy.

LA-ICP-MS (laser ablation inductively coupled plasma mass spectrometry): In situ technique using a laser beam to scavenge the sample surface to analyze its elemental or isotopic composition with a high sensitivity. Typical lasers drill holes of 10–20 μm in diameter. Cannot analyze H, N, and O.

μ-CT (x-ray computed micro tomography): Radiographic imaging technique that uses x-ray to analyze the internal structure of materials. It can be used to produce 3D images at a spatial resolution >1 μm.

XPS (x-ray photoelectron spectroscopy): Surface analysis technique providing information on elemental composition and chemical state of surfaces (<5 nm depth). The lateral spatial resolution is < 7.5 μm.

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