

Review Article

Nano-Scale Secondary Ion Mass Spectrometry: A Paradigm Shift in Soil Science

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Soils exhibit structural heterogeneity across diverse spatio-temporal scales, yielding myriad of microhabitats, highlighting the need for a nuanced understanding of the intricate interactions within the soil matrix. At the nanometer scale, the interplay among organic matter (OM), mineral particles, and microbiota intricately govern the long-term destiny of soil carbon (C), nutrient cycling, and the fate of both organic and inorganic pollutants. Notably, the sorption of soil organic matter (SOM) onto smaller clay particles and its entrapment in microaggregates further contribute to this complex dynamic. Understanding these processes depends on recognizing their scale-dependent nature, necessitating sophisticated techniques for investigation. Although various methods are employed across scales, the current set of techniques still lacks the requisite sensitivity and resolution for microscale data collection. To address this limitation, the adoption of novel microscopic and spectroscopic techniques capable of probing molecular, isotopic, and elemental patterns at the micro to nano scale becomes imperative. Among these cutting-edge methodologies, the nano-scale secondary ion mass spectrometer (NanoSIMS) emerges as a paradigm-shifting tool. Representing the latest evolution in ion microprobes, NanoSIMS seamlessly integrates high-resolution microscopy and isotopic analysis, maintaining unparalleled signal transmission and spatial resolution, reaching as fine as 50 nm. Its capabilities extend beyond conventional applications in science, as evidenced by recent breakthroughs in utilizing NanoSIMS to study biophysical interfaces in soils. This article underscores the pressing need to advance the incorporation of NanoSIMS as a pioneering instrumentation technique in soil studies. Furthering the implementation of this novel instrumentation technique in soil studies will pave avenues and aid in the advancement of future research.

1. Introduction

Soils constitute a fundamental component of the ecosystem, embodying a complex amalgamation of organic and inorganic constituents intricately intertwined. The organic phase comprises partially recalcitrant substances derived from plant litter, faunal biomass, and microbial activity [1]. Simultaneously, the major inorganic components, encompassing quartz, clay minerals, and oxides/hydroxides of iron (Fe), manganese (Mn), and aluminium (Al), along with carbonates, contribute to the soil matrix. During the process of soil formation, these components interact, giving rise to primary soil particles that coalesce into microaggregates. Subsequently, these microaggregates undergo additional stabilization through short-term binding agents such as roots and hyphae, culminating in the formation of macroaggregates [2]. Consequently, soils exhibit structural heterogeneity across various spatial and temporal scales [3, 4], with the processes governing the stabilization of OM occurring at the submicron scale [5].

The distinctive binding characteristics of organic components in soil, coupled with the adsorption capacities of soil minerals, propel the creation of complex organo-mineral assemblies, such as microaggregates [2]. These associations play a pivotal role in stabilizing C against degradation, representing a pathway for C sequestration, a critical challenge in mitigating climate change [6]. Moreover, soils host a vast microbial biodiversity comprising bacterial, archaeal, and fungal taxa, crucial for driving biogeochemical cycles. The micro-level structuring of soils generates a variety of microenvironments that impose selective influences on the microbiome, playing a pivotal role in preserving and sustaining soil microbiome diversity [7, 8].

Microbial communities mediate a myriad of soil reactions, and their interplay at small scale with the physical, chemical, and biotic elements of the soil environment regulates these reactions [9, 10]. However, understanding the intricate biogeochemical processes in the rhizosphere requires observations at the junction of soil, root cells, and microorganisms, necessitating investigations at the cellular scale [11–13].

Furthermore, the substantial hazard of heavy metal (HM) contamination in soils extends globally, emanating from agricultural, industrial, and mining activities [14, 15]. Excessive accumulation of HM in soils has far-reaching consequences for food safety, human well-being, and ecosystem functioning [16, 17]. The complex nature of soils, comprising minerals, OM, microorganisms, and other solid components, determines the cycling activities of HMs [18].

Comprehension of the dynamics of HMs at composite junctions is crucial for formulating ecological risk assessments and effective pollution remediation [19, 20]. The interactions among all the above mentioned components are scale-dependent (Table 1), posing a challenge to

understanding their behavior. The insufficiency of sensitive techniques for data collection at the requisite scales hampers progress in unraveling these complex interactions. Therefore, to address these challenges, novel spectroscopic techniques are essential for observing submicron-sized organo-mineral associations, aggregate interiors, C transfer, microbial ecology, and pollutant determination. The advent of a new era in ion microprobes, specifically NanoSIMS, presents a breakthrough in this regard.

NanoSIMS facilitates precise, spatially explicit analysis of elemental and isotopic composition with a resolution reaching 50 nm. Widely utilized in soil studies, NanoSIMS employs a focused beam of ions to produce secondary ions from the surface of the sample, facilitating high visualization and isotopic characterization [21]. Its applications span SOM dynamics, soil-root interactions, microbial ecology, and metal behavior, providing substantial information about the intricate associations that govern soil health, fertility, and nutrient cycling [22–24].

The objectives of this paper were (i) to delve into the principles and features of NanoSIMS, offering insights into practical considerations of the instrumentation; (ii) to provide a thorough summary of applications of NanoSIMS in various facets of soil studies; (iii) the integration of NanoSIMS with other techniques; (iv) review the challenges and opportunities associated with NanoSIMS, emphasizing its potential to propel our comprehension of soil functioning at the sub-micron scale; (v) aims to enhance the current understanding in the field by highlighting recent progress and suggesting pathways for innovative research, intending to improve our comprehension of the complex interplay among components of soil and micro-interface processes.

1.1. Methodology Used to Conduct Review Process. In this study, we used a comprehensive methodology to analyze the diverse areas of NanoSIMS research, primarily revolving on its employment within soil science investigations. Exploiting distinct scholarly databases including Google Scholar, Springer Link, Web of Science, and Scopus, we conducted a systematic search using keywords such as “NanoSIMS,” “SIMS,” “soil science,” and “microbiome.” Our search strategy aimed to identify peer-reviewed literature encompassing research articles, review papers, and book chapters pertinent to NanoSIMS applications in soil studies. A qualitative assessment was performed on the acquired literature, with a focus on articles published between 2000 and 2023, thereby capturing contemporary development in the field while excluding pre-2000 publications. A total of 145 relevant publications were chosen for in-depth analysis, forming the basis of our review. The systematic layout of subsequent sections is delineated in Figure 1.

TABLE 1: Biological processes and methodologies at various spatial scales [21].

Processes	Physical scale	Techniques
		Gas fluxes
		SOM fractionation
	cm	Microbial biomass
		Inorganic nutrients
	mm	
	Roots	
	Aggregates	Molecular techniques
	Sand	
	μm	Computer-aided X-ray tomography
Microbe-plant nutrient transfer	Silt	Mass spectrometry
	Clay	Microsampling/microscopy
Nutrient cycling	nm	NanoSIMS

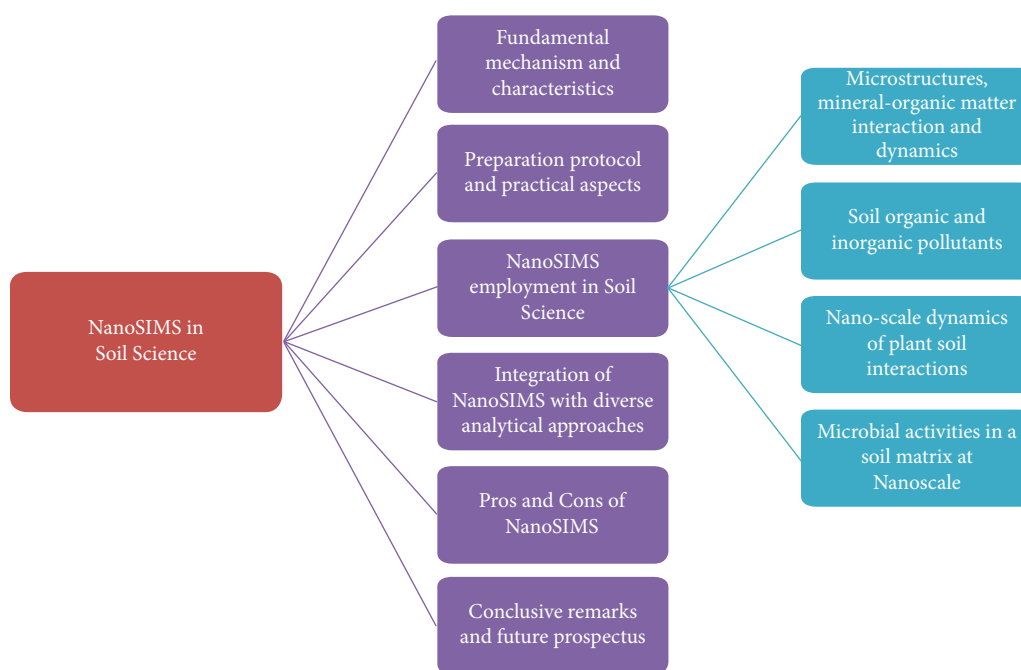


FIGURE 1: Systematic layout of the subsequent sections.

2. Fundamental Mechanism and Characteristics of NanoSIMS

The evolution of secondary ion mass spectrometry (SIMS) has culminated in the development of NanoSIMS, a sophisticated and high-resolution spectroscopic technique [25]. Distinguished by its ability to integrate high-resolution microscopy and isotopic analysis, NanoSIMS delivers spatially specific data pertaining to the molecular and isotopic compositions of materials [26, 27]. This instrument deploys a precise primary beam of ions and facilitates the efficient extraction of secondary ions (ions extracted from the sample), which are subsequently mass-analyzed (separation of extracted ions based on mass to charge ratios) to yield information with exceptional spatial resolution [26, 28]. The optical configuration and segmentation of NanoSIMS are illustrated in Figure 2, incorporating novel techniques that

yield significantly more data and offer spatial information in three dimensions regarding various characteristics of the sample. NanoSIMS analysis is inherently destructive, wherein the sample undergoes continual bombardment with robust ion sources (Cs^+ and O^-), a process known as sputtering (vaporization of solid material by ion energy). The charge of the generated secondary ions is contingent upon the primary ion beam used. For instance, Cs^+ and O^- ion beams produce negative and positive secondary ions, respectively [24, 29, 30]. Subsequently, the extracted secondary ions are accelerated and directed to the magnetic sector mass analyzer, where they are separated based on mass-to-charge ratios [26]. This process generates spatially referenced spectra, allowing the creation of maps for nearly any selected atomic mass [25]. By employing a primary beam to bombard the sample and examining the resultant secondary ions, NanoSIMS has the capability to identify concentrations

ranging from trace isotopes to individual bacteria in the order of mg/kg, facilitating the exploration of micro-site heterogeneity within soil.

NanoSIMS possesses noteworthy attributes, including superior lateral resolution (less than 50 and 200 nm for Cs^+ and O^- ions, respectively) and remarkable sensitivity. It can distinguish isotopes with similar atomic masses, such as $^{13}\text{C}^-$ and $^{12}\text{C}^1\text{H}^-$, as well as $^{12}\text{C}^{15}\text{N}^-$ and $^{13}\text{C}^{14}\text{N}^-$. The instrument can set up to 7 signal detectors, simultaneously measuring seven varieties of elements or isotopes with elevated analytical accuracy, covering the elemental range from hydrogen to uranium [31, 32]. Consequently, scientists can simultaneously observe various elements and isotopes in a specimen, augmenting the understanding of the interactions, makeup, and distribution of distinct soil constituents. The NanoSIMS apparatus generates information presented as 2 or 3-dimensional images, depicting the arrangement of distinct sample elements [32–34]. The advancement in the field of analytical precision and improved quality of image processing has additionally enhanced correction, area of interest segmentation, and predictions related to chemical composition [35, 36]. The concluding stage in the procedure of NanoSIMS analysis encompasses the interpretation of obtained data to derive informative outcomes [35, 37]. Ultimately, NanoSIMS contributes in advancing our comprehension of the 3-dimensional structural layout of the soil matrix, microbiome assemblages, and the cycling of nutrients. The employment of techniques such as stable isotope labeling and the NanoSIMS particle analysis allows for the documentation of elemental and isotopic patterns within sub-micrometer-sized soil particles, promoting the investigation of the biogeochemical cycling of SOM [38–40].

2.1. Preparation Protocols and Practical Aspects in Sample Handling. Advancements in technology have significantly improved our understanding of soil processes on a small scale. However, incorporating NanoSIMS into soil studies presents various practical challenges that must be addressed. The initial and formidable task involves sample size and preparation for NanoSIMS analysis. Effective preparation of the sample is vital and encompasses methods like laser ablation, chemical extraction, and mechanical grinding [41]. Meanwhile, the interpretation of acquired data heavily relies on the methods of data acquisition and analytical techniques [42, 43].

NanoSIMS analysis necessitates samples to be devoid of moisture, possess stability, conductivity, and resilience to high vacuum conditions (NanoSIMS operates at ultra-high vacuum), and have a flat and highly polished surface to minimize the charging effect that may occur during analysis (Figure 3(a)). In order to keep high vacuum of the analysis chamber, NanoSIMS cannot analyze liquid or volatile samples, therefore, the sample should be nonvolatile. In addition, previous studies have revealed that both the precision and accuracy of SIMS were affected by smoothness of the sample surface. Therefore, to obtain accurate results, polishing is required. Furthermore, the charging occurs

when an ion beam is used on non-conductive materials, thus causing irradiation which leads to an accumulation of electrostatic charges on the specimen's surface. This build-up of charge can make imaging and chemical analysis of the sample challenging. Therefore, combining gold coating (conductive layer on the sample surface) with an electron flood gun can help reduce the charging effect (Figure 3(b)). The sample must be <4 mm thick and 10 mm/13 mm/25 mm size in a disc shape [3]. Figure 3(c) provides a visual representation of the sample holder configuration along with the diverse sample holders. The employment of resin-based methods is common for exploring the intricate distribution of microorganisms at a fine scale within soil [45–47]. NanoSIMS has the capability to simultaneously identify and measure up to 5 or 7 ion species, enabling the concurrent assessment of 2 to 5/7 isotopes originating from the same analysed material. This functionality is essential, particularly for samples vulnerable to damage from the primary ion beam, considering the inherently destructive nature of NanoSIMS analysis. Nevertheless, there are constraints when simultaneously analyzing ion species, where the separation of peaks is confined by the physical dimensions of detectors and the magnetic field, and beyond mass 30, simultaneous analysis becomes challenging [3].

NanoSIMS stands out due to its capacity to function with elevated mass resolution, all the while preserving remarkable signal transmission and spatial resolution [26]. Optimal adjustments are imperative to ensure effective differentiation of isobars during the analysis. For instance, mass 26 might encounter overlaps from $^{12}\text{C}^{14}\text{N}$, $^{13}\text{C}_2$, and $^{12}\text{C}^{13}\text{C}^1\text{H}$, introducing the possibility of interference with the targeted ion species. This underscores the need for a substantial mass resolving power to achieve adequate separation of peaks [3]. Furthermore, the naturally confined operational field of view for the NanoSIMS instrument typically ranges between 5 and 50 μm . Herrmann et al. [3] found that the highest practical field of view/ion image was about $30 \times 30 \mu\text{m}^2$, and beyond this range, noticeable distortions were observed at the edges. Consequently, meticulous techniques need to be formulated to determine the sample position for NanoSIMS analysis. Utilizing high-resolution microscopic visualization enables the identification of relevant features necessary for pinpointing regions of interest for NanoSIMS probing. However, achieving this is especially demanding given the minute spatial scales involved at the nano-level. The NanoSIMS system incorporates an optical microscope coupled with a CCD camera and a secondary electron detector to aid in sample navigation (see Figure 2). Combining NanoSIMS with other existing techniques becomes essential to enhance the precision of the analysis.

2.2. NanoSIMS Employment in Soil Science: Exploring New Dimensions. NanoSIMS proves to be a versatile tool in soil science, significantly advancing our comprehension of crucial soil processes (Table 2). Its exceptional sensitivity enables the measurement of trace elements and isotopes, along with the visualization of nutrient and SOM patterns at the microscopic level. This proficiency offers novel insights

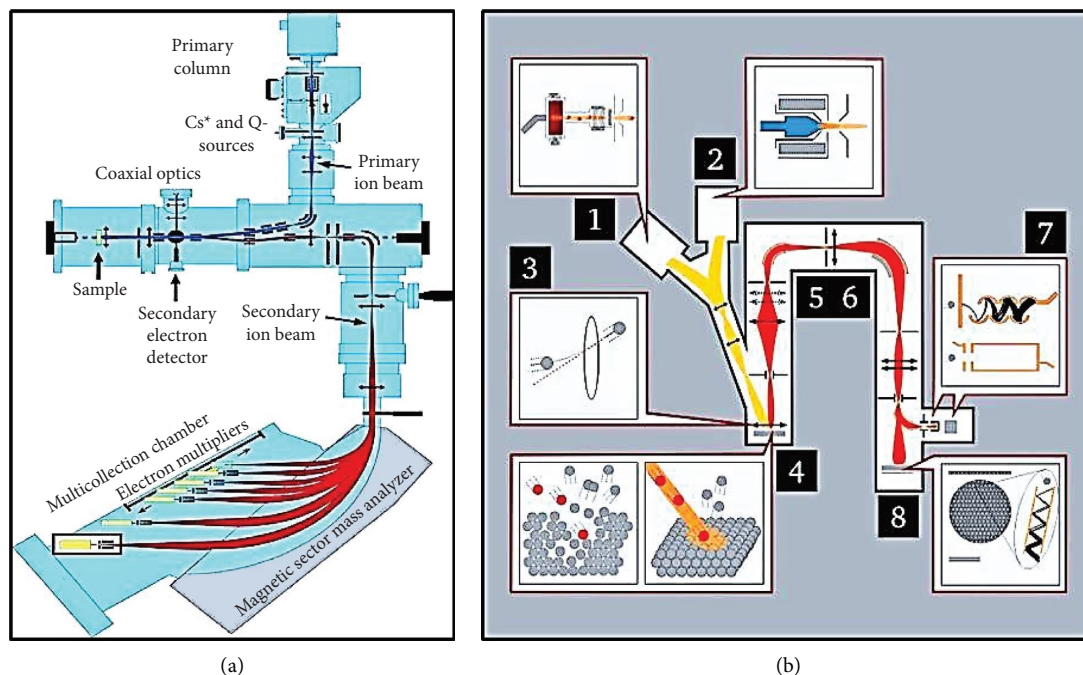


FIGURE 2: (a) Diagrammatic representation of NanoSIMS ion optics of cama NanoSIMS 50 L (b) the illustration portray a typical dynamic SIMS apparatus, wherein energetic ions emitted from an ion gun (1 or 2) are targeted onto the sample (3), induces ionization and removal of surface atoms through sputtering (4). Subsequently, the secondary ions are collected by ion lenses (5) filtered based on atomic mass by mass analyzer (6), and directed towards an electron multiplier (7, upper part), a faraday cup (7, lower part), or a CCD screen (8), then generates information as 2 or 3-dimensional images, depicting the arrangement of distinct sample elements [26, 27].

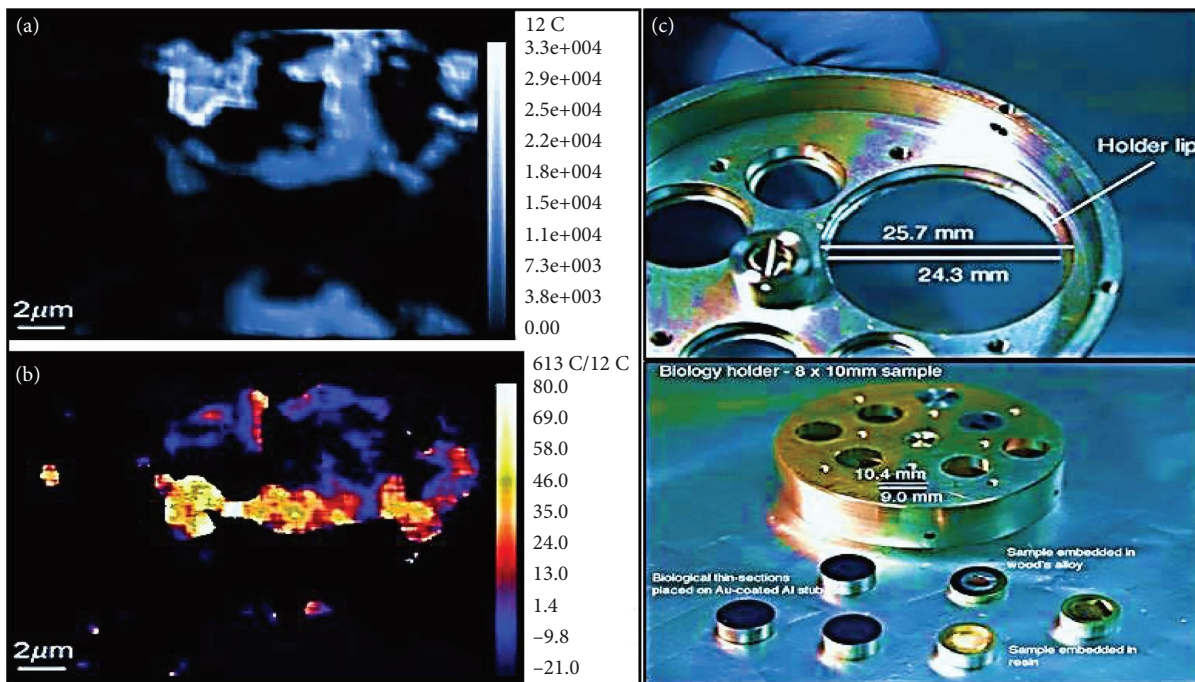


FIGURE 3: Soil aggregates derived from a sandy soil enriched with ^{13}C and ^{15}N labeled pinus ponderosa fine roots and needles [44]. In (a), a NanoSIMS ^{12}C image displays a 15 mm field of view of an uncoated soil aggregate, dried, and pressed into an aluminium stub. (b) illustrates a NanoSIMS $^{13}/^{12}\text{C}$ image of the same region, coated with gold and utilizing the electron flood gun. The image is a compilation of 50 individual 256×256 pixel planes (scans), with the scale on (b) indicating $^{13}/^{12}\text{C}$ ratio values. Panel (c) provides information on the sample holder configuration and various sample holders.

TABLE 2: Summarizing several soil science studies employing NanoSIMS from 2005 to 2023 to emphasize advancements in the field.

S. no.	Country	Title of the study	Parameters studied/outcome	Authors
1	Germany	“Elucidating soil structural associations of organic material with nano-scale secondary ion mass spectrometry (NanoSIMS)”	A carefully designed experiment was conducted, focusing on primary soil particles derived from the fine silt and clay fractions, along with soil aggregates measuring less than 6.3 μm . The study involved two distinct soil types, namely an Albic Luvisol and a Haplic Chernozem. In this investigation, a tracer composed of a blend of amino acids labeled with ^{13}C and ^{15}N was employed	[48]
2	Germany	“Submicron scale imaging of soil organic matter dynamics using NanoSIMS -from single particles to intact aggregates”	An experimental study was executed in the laboratory, employing soil particles sourced from the fine silt and clay fractions of an Albic Luvisol. In addition, occluded particulate organic material and intact soil aggregates from a Haplic Chernozem were incorporated into the experimental setup. The inquiry utilized a tracer comprising a blend of amino acids labeled with ^{13}C and ^{15}N	[49]
3	USA	“Nanoscale investigation of the association of microbial nitrogen residues with iron (hydr)oxides in a forest soil O-horizon”	The objective was to document the initial micro-scale changes associated with introducing chitin labeled with ^{13}C - and ^{15}N into environments predominantly influenced by fungi within the O-horizons of ancient forest soils	[50]
4	Germany	“A NanoSIMS study on the distribution of soil organic matter, iron and manganese in a nodule from a stagnosol”	Investigated the spatial allocation of Fe and Mn in stagnosols, with a specific focus on their interaction with OM NanoSIMS for submicrometer-scale spatial resolution	[51]
5	France	“NanoSIMS investigation of glycine-derived C and N retention with soil organo-mineral associations”	Assessed the surface allocation of ^{13}C and ^{15}N derived from glycine in particles randomly extracted from soil density fractions through the application of NanoSIMS	[52]
6	Australia	“Advancement in the application of NanoSIMS and Raman microspectroscopy to investigate the activity of microbial cells in soils”	Devised an optimized procedure for efficiently extracting cellular extracts from soils, streamlining the process to enable subsequent application of single-cell methods	[53]
7	France	“Nanoscale evidence of contrasted processes for root-derived organic matter stabilization by mineral interactions depending on soil depth”	The objective of this investigation was to explore the fate of root material labeled with ^{13}C and ^{15}N at depths of 30 and 90 cm following a three-year incubation period. Furthermore, the study sought to elucidate the characteristics of organic matter stabilized through interactions with metal oxides	[54]
8	Australia	“Nitrogen-rich microbial products provide new organo-mineral associations for the stabilization of soil organic matter”	Employing NanoSIMS, this investigation scrutinized changes in C and N within a vertisol and an Alhisol following a 365-day incubation with pulse-labeled lucerne (<i>Medicago sativa</i> L., enriched with ^{13}C and ^{15}N). The primary objective was to differentiate newly introduced organic matter from the pre-existing SOM	[55]
9	China	“NanoSIMS measurement of sub-micrometer particles using the local thresholding technique”	To enhance recognition rates, this study has devised a high-efficiency sub-micron particle recognition method	[34]
10	United States	“Complexation by organic matter controls uranium mobility in anoxic sediments”	Harnessing advanced nanoscale imaging techniques, specifically NanoSIMS and scanning transmission X-ray microscopy (STXM), in conjunction with a density-based fractionation method, this study systematically and microscopically separated organic and mineral components from alluvial sediments contaminated with uranium	[56]
11	Australia	“Zinc and cadmium mapping by NanoSIMS within the root apex after short-term exposure to metal contamination”	Validation of NanoSIMS as an effective instrument for spatial identification and visualization of specific ultra-trace metal isotopes, such as ^{70}Zn , within actively developing root tips	[57]
12	Australia	“Soil organic matter is stabilized by organo-mineral associations through two key processes: The role of the carbon to nitrogen ratio”	Incorporation and fixation of residues labeled with ^{13}C and ^{15}N derived from lucerne (<i>Medicago sativa</i>) and buffel grass (<i>Cenchrus ciliaris</i>)	[58]

TABLE 2: Continued.

S. no.	Country	Title of the study	Parameters studied/outcome	Authors
13	China	“Characterization of Cu distribution in clay-sized soil aggregates by NanoSIMS and micro-XRF”	Deepening our understanding of the interplay between HMs and various constituents within the soil	[59]
14	Australia	“Soil organic matter is stabilized by organo-mineral associations through two key processes: The role of the carbon to nitrogen ratio”	Leveraging NanoSIMS, this investigation delved into the amalgamation and fixation of residues labeled with ^{13}C and ^{15}N originating from lucerne (Medicago sativa) and buffel grass (Cenchrus ciliaris) throughout the incubation period within a vertisol situated in temperate Australia	[58]
15	China	“Unveiling of active diazotrophs in a flooded rice soil by combination of NanoSIMS and $^{15}\text{N}_2$ -DNA-stable isotope probing”	NanoSIMS was employed to analyze the ^{15}N enrichment of soil DNA within stable isotope probing fractions	[60]
16	Germany	“Porosity and organic matter distribution in jarositic phyto tubules of sulfuric soils assessed by combined μCT and NanoSIMS analysis”	This study employed a combination of X-ray computed microtomography (μCT) and NanoSIMS to examine jarositic phyto tubules, aiming to elucidate porosity and the spatial distribution of OM at the scale most relevant for microbial processes	[61]
17	China	“Visualizing mineral-associated organic matters in long-term fertilization treated soils by NanoSIMS and SR-FTIR”	Extended manure fertilization elevated the ratios of $^{27}\text{Al}^{16}\text{O}^-/^{12}\text{C}^-$, $^{27}\text{Al}^{16}\text{O}^-/^{12}\text{C}^{14}\text{N}^-$, $^{56}\text{Fe}^{16}\text{O}^-/^{12}\text{C}^-$, and $^{56}\text{Fe}^{16}\text{O}^-/^{12}\text{C}^{14}\text{N}^-$ in comparison to scenarios with no fertilization and chemical fertilization	[62]
18	Germany	“Microscale carbon distribution around pores and particulate organic matter varies with soil moisture regime”	Examining the influence of soil moisture conditions on microscale C gradients using an inventive mapping procedure to analyze particulate organic matter and C distribution within the soil matrix	[63]
19	Germany	“Iron (hydr)oxide formation in Andosols under extreme climate conditions”	Assessed the ecological significance of terrestrial Fe cycling by examining the pathways of Fe-(hydr)oxide transformation in acidic topsoils of south patagonian Andosols derived from rhyolitic tephra	[64]
20	Canada	“Enhanced loss but limited mobility of pyrogenic and organic matter in continuous permafrost-affected forest soils”	Examined the vertical mobility, allocation in soil fractions, and decomposition losses of extensively ^{13}C -labeled pyrogenic organic matter along with its precursor ryegrass organic matter	[65]

into the dynamics of nutrient absorption and turnover [31, 66]. NanoSIMS applications span a wide range, delving into the exploration of SOM dynamics and cycling, contributing innovative perspectives on the makeup, spatial patterns, and micro-scale turnover of SOM. Furthermore, NanoSIMS contributes to understanding nutrient cycling and soil fertility [31, 67]. Exploring soil-root interactions, NanoSIMS sheds light on how essential nutrients like nitrogen (N), phosphorus (P), and sulfur (S) are distributed and taken up by roots, which, in turn, deepens our comprehension of mechanisms involved in nutrient uptake and plant-soil interactions [68–70].

In addition, NanoSIMS serves as a valuable instrument for evaluating the behaviour of metals in soils, sensing trace elements even at exceedingly low concentrations. This capacity delivers crucial data for the examination of metal distribution in soil, their accessibility to plants, and their influence on soil fertility and plant growth [19, 24]. The insights gained makes a substantial contribution to our comprehension of how metals behave in soil and their potential ecological ramifications [19, 24, 71, 72]. The potential applications of NanoSIMS are individually discussed below:

2.3. Microstructures, Mineral-Organic Matter Interactions and Dynamics. NanoSIMS is indispensable in soil science studies, primarily regarding micro-structure and the mineral and organic matter interactions. This requirement rises because of its unparalleled ability to provide high-resolution imaging and quantitative elemental analysis at the nano-scale. In the complex microstructure of soils, NanoSIMS enables the visualization and characterization of individual mineral particles, microbial cells, organic compounds, and their spatial arrangements with outstanding detail.

NanoSIMS imaging offers a promising avenue for studying the mechanisms governing mineral-organic matter interactions, micro-scale soil architecture, and soil biogeochemistry at a scale previously unattainable with conventional methods. The foundational work of Kubiena [73] marked a systematic approach to in situ soil feature exploration. This involved the use of intact soil clods infused with epoxy resin, allowing for thin sectioning and subsequent examination via transmitted light microscopy. The advent of analytical techniques like transmission electron microscopy (TEM), atomic force microscopy (AFM), and NanoSIMS has triggered a renaissance in micromorphological soil examination. Nevertheless, challenges arise as mineral particles hinder experiments involving elemental mapping and isotope tracing within intact soil matrices, mainly due to difficulties in embedding, thin-sectioning, and potential electrical charging effects [74]. Despite these challenges, proof-of-concept studies have successfully demonstrated the imaging of ^{15}N and ^{13}C isotope additions in 2-dimensions using NanoSIMS within both natural or synthetic soil matrices, providing insights into micro-scale heterogeneities and microbial activity [3, 49, 50, 75].

NanoSIMS has been pivotal in pushing forward our comprehension of microscale dynamics of SOM and the cycling of nutrients. This analytical technique has contributed significantly to unraveling the spatial arrangement, makeup, and SOM transformation [63, 76], shedding light on SOM formation and degradation mechanisms. NanoSIMS has proven instrumental in characterizing individual molecules within SOM, discerning its origins, and elucidating soil C cycling [65, 77]. The distinctive ability of the technique in isotope imaging, exemplified by Herrmann et al. [3], where NanoSIMS successfully identified isotope-enriched bacterial cells in silty soil abundant in ^{15}N , underscores its excellence in exploring biophysical interactions within soil on a smaller scale. Additionally, NanoSIMS possesses the capacity to produce isotope maps, enabling the monitoring of labeled compounds and providing understanding into the interactions among clay minerals, OM, and Fe oxides at the nano-scale.

Explorations into mineral-organic associations have traditionally relied on bulk analysis methods applied to operationally defined physical fractions [78, 79]; however, these techniques are inefficient to provide fine-scale information. In contrast, NanoSIMS presents an opportunity to examine organo-mineral assemblages within intact spatial structures. Leveraging stable isotope labeling experiments, NanoSIMS images can unveil the dispersion pattern and dilution of tracer materials as they penetrate the soil matrix [75]. This approach also enables the investigation of whether certain types of OM predictably associate with specific mineral phases.

The unique capacity of NanoSIMS in achieving high mass and lateral resolution allows the mapping of both elemental and isotopic distributions, as demonstrated by Heister et al. [1] in a study of artificial soil mixtures, by studying the distinct derived ions of organic material ($^{12}\text{C}^-$ and $^{12}\text{C}^{14}\text{N}^-$) and minerals ($^{28}\text{Si}^-$, $^{27}\text{Al}^{16}\text{O}^-$, and $^{56}\text{Fe}^{16}\text{O}^-$). In the context of this research, NanoSIMS served as a tool for conducting microscale elemental mapping of OM on mineral surfaces. The findings revealed a distinct pattern where OM exhibited an inclination to adhere to phyllosilicate clays in discrete patches, while small ferrihydrite particles were enveloped by continuous coatings of OM [1]. Such nuanced variations at the microscale would have eluded detection through SEM/EDX measurements.

Another illustrative case involves the work of Mueller et al. [49]. In their study, which employed resin-embedded soil macroaggregates, they discerned diverse isotopic enrichment at the microscale subsequent to application of $^{13}\text{C}/^{15}\text{N}$ label to natural soils. Speculation arose regarding the increased utilization of freshly added OM due to microbial activity, or the potential scenario where various soil components exhibit distinct sorption capacities. The distinctive capability of NanoSIMS's in identifying stable isotope tracers at the microscale played a pivotal role in confirming the anticipated physical dimensions of organo-mineral associations in both studies.

Remusat et al. [75] employed a comparable methodology to visualize intact soil particles that displayed minimal levels of isotopic enrichment. These samples were collected from a temperate forest 12 years following a ^{15}N litter labeling experiment. Their observations delineated microenvironment characterized by isotopic enrichment, referred to as “ ^{15}N hot spots,” predominantly located on mineral surfaces. Within one such microsite, the authors suggested a potential association between ^{15}N enrichment and the existence of microbiome metabolites. This holistic strategy, amalgamating NanoSIMS with alternative microscopic methods like scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX), STXM/near edge X-ray absorption fine structure (NEXAFS) holds significant promise as a lucrative avenue to deduce the spatial and molecular fate of marked organic materials within a matrix. Furthermore, this comprehensive strategy holds the promise of significantly enhancing our understanding of the mechanisms involved in sorption, occlusion, and decomposition processes occurring at finely resolved scales.

Moreover, Kopittke et al. [80] examined the influence of extrinsic expansion of C and N through a labeling experiment in Vertisols and Alfisols. They employed NanoSIMS to discern variations in mineral confinement between lately introduced and the initial SOM. Over the course of 365 days, the study revealed a preference of N-rich products to attach themselves to mineral particles. The observation implies the possibility that these products could give rise to novel organo-mineral settings on the C-free mineral surfaces, likely contributing to an enhanced storage of OC.

Numerous investigations employing NanoSIMS have offered valuable understanding into the influence of mineral protection on the fixation and transformation of OM at the submicron level [40, 81–83]. Within the natural soil matrix, NanoSIMS discoveries revealed the co-localization of indigenous and recently introduced C with minerals, supplying direct evidence that these minerals may act as favored locations for preserving labile C [84]. Additionally, the introduction of ^{44}Ca into soils stimulated microbiome activities and fostered connections among mineral and byproducts of microbes, emphasizing the function of Ca as a mediator in the intermixed biotic-abiotic soil organic carbon (SOC) cycling [85]. While notable procedural challenges persist, these examples underscore the potential benefits of well-designed experiments utilizing NanoSIMS data to unravel microbiological and chemical processes within soil niches. Conclusively, NanoSIMS serves as a crucial tool for advancing our understanding of soil processes and their implications for ecosystem functioning and environmental sustainability.

2.4. Soil Organic and Inorganic Pollutants. Soil pollution, or contamination, arises from the inclusion of hazardous compounds into soil ecosystem, originating from numerous sources such as agricultural practices, improper waste disposal, industrial activities, and urbanization [14]. In agriculture, soil functions as the cornerstone for crop production, providing necessary nutrients, improving water

retention, and enhancing structural support for plant growth. However, contaminated soils pose substantial harm to crop health and productivity, leading to reduced crop output and economic crises. Furthermore, soil pollution has the potential to disturb soil ecosystems and biodiversity, thereby precipitating enduring ecological deterioration. Soil pollution, arising from both organic and inorganic pollutants, poses a significant threat to global rice production in agriculture. HM contamination, originating from industrial, agricultural, and mining activities, stands out as a major concern [15]. The detrimental effects of the undue accumulation of HM in soils have far-reaching ramifications on food safety, human well-being, and the ecological services provided by the soil. Inorganic pollutants, particularly metals and metalloids like lead (Pb) and arsenic (As), including radioactive particles from nuclear incidents, contribute to soil complexity and challenge soil scientists in understanding their interactions [86–88]. Soils, intricate ecosystems composed of minerals, OM, microorganisms, and various solid components, feature inorganic and organic clay particles critical to HM cycling activities [89]. A profound comprehension of HMs at composite interfaces becomes imperative for conducting accurate environmental risk assessments and implementing effective pollution remediation measures [19, 20].

Notably, SIMS has been employed to scrutinize the fine-scale patterns of metalloids, metals, halogens, and organic pollutants in various organisms, plants, animals, and human tissues [90–98]. The advanced analytical technique NanoSIMS, an extension of SIMS, proves invaluable in studying metal behavior and distribution in soils [57, 66, 71]. NanoSIMS, with its high spatial resolution, facilitates in-situ imaging of metals, such as plutonium, providing insights into metal-soil component interactions and transport mechanisms [99, 100]. The technique enables precise observations of spatial relationships between OM and inorganic contaminants, offering the potential to assess particle “hot spots” at micron level [101].

Studies [102–104] showcase the versatility of NanoSIMS in exploring interactions of elements like As, Fe oxides, and OM in different soil environments. These investigations reveal variations in metal distributions among different regions, emphasizing the role of specific soil components in the availability and uptake of metals. The unique insights provided by NanoSIMS extend to the micro-scale, revealing variations in the metal distribution like copper (Cu), among active soil components of different sizes, underscoring the importance of understanding metal-soil component associations [19, 59].

In essence, NanoSIMS stands as a potent tool, furnishing invaluable insights into the molecular-level and nano-scale element attributes within natural soils. Its remarkable attributes, such as high spatial resolution, multi-element, and isotope analysis capabilities, along with compatibility with stable isotope labeling techniques, offer distinctive perspectives concerning interplay of nutrient cycling, SOM, and microbiome functioning. Despite its restricted scanning area compared to techniques like micro-XRF, NanoSIMS delivers meticulous information about the form, distribution,

mobility, and destiny of elements. This precision facilitates an enhanced comprehension of soil functions in the environment, encompassing their influence on plant growth, C and nutrient cycles. By unveiling intricate interplay and processes at these minute scales, NanoSIMS makes a substantial contribution to our in-depth understanding of the ecosystem functioning.

2.5. Nano-Scale Dynamics of Plant-Soil Interactions.

Understanding root-soil interactions is critical for unveiling the complex mechanisms governing plant growth and soil health. The root-soil interface (rhizosphere) and the fungal hypha-mineral interaction zone (hyphaesphere) are dynamic zones crucial for mineral weathering [105]. Soil-root interactions are fundamental for plant growth, involving intricate processes such as nutrient absorption, ion transport, and microbial-root interactions [106, 107]. By investigating root-soil interactions, researchers can reveal how plants adapt to diverse soil conditions, optimize resource utilization, and respond to environmental extremes like drought, salinity, and nutrient deficiency. Despite their significance, studying these interfaces is challenging due to soil opacity and potential disturbances affecting plant growth and rhizospheric processes. The limitations of conventional techniques like radiotracer imaging and fluorescent labeling have prompted the exploration of advanced technologies such as NanoSIMS [108, 109]. Positioned between light microscopy and X-ray techniques, NanoSIMS facilitates high-resolution, noninvasive measurements, bridging the gap in understanding the transfer of C and nutrients among soil, roots, and microbes. NanoSIMS studies, exemplified by Clode et al. [110] and Rumpel et al. [54], have provided submicron-level insights, detecting and imaging ^{15}N compounds within single microbes present in both root and matrix. Rumpel et al. [54] utilized a 16 keV primary Cs^+ ion beam for the acquisition of secondary ions, specifically ^{12}C , ^{13}C , ^{16}O , ^{26}CN , and ^{27}CN , employing multicollection mode. Conversely, Clode et al. [110] employed a Cs^+ primary ion probe with impact energy of approximately 16 keV, applying a beam current ranging from 1 to 2 pA. The primary ion beam was focused to achieve a diameter of approximately 100 nm. Concurrently, the secondary ions $^{12}\text{C}^-$, $^{12}\text{C}^{14}\text{N}^-$, $^{12}\text{C}^{15}\text{N}^-$, and $^{28}\text{Si}^-$ were recorded across masses 12, 26, 27, and 28, respectively. The findings revealed depth-dependent processes, suggesting varied interactions between decomposed plant material, metal oxides, and OM at different soil depths.

NanoSIMS has played a crucial role in advancing our comprehension of soil-root interactions by enabling high precision isotope imaging in biological specimens. Studies by Vidal et al. [111] and Fang et al. [112] exemplify NanoSIMS' role in monitoring the destiny of marked root secretions and assessing positional fluctuations in Fe/P ratios near rice roots, respectively. The presence of roots enhances mineral dissolution and availability, resulting in the formation of minerals at the nano scale. This process also supports the development of minerals characterized by short-range ordering, facilitated by the secretion of

substances from the roots [113]. Additionally, NanoSIMS provides insights into the interplay between roots and microbiome, the spatial nutrient arrangement, and the absorption of nutrients. Notably, it revealed plants' ability to deliberately attract particular groups of microorganisms to their roots, influencing a substantial impact on the overall growth of plants [114, 115]. While NanoSIMS has enhanced knowledge of specific facets concerning the interactions between roots and soil, further research integrating NanoSIMS with complementary techniques like synchrotron radiation X-ray fluorescence and X-ray absorption spectroscopy is crucial. This interdisciplinary approach is necessary for a comprehensive understanding of the complex dynamics involving plants, microorganisms, and soil, all of which collectively play pivotal roles in shaping the growth of plants. Addressing these gaps will allow for a mechanistic understanding of physical, chemical, and biological mechanisms unfolding across diverse spatio-temporal scales in the plant-microbe-soil continuum, paving the way for more effective strategies in agriculture and environmental science [116].

2.6. Microbial Activities in a Soil Matrix at Nano-Scale.

Microbes play important functions in numerous biogeochemical processes within soils, such as the decomposition of OM, mineralization of nutrient, nitrogen fixation, and the cycling of carbon, nitrogen, and other essential elements. These activities are pivotal for balancing soil fertility, supporting plant growth, and sustaining health of ecosystem. Therefore, investigating microbial activities in soil matrices at the nano-scale is of extreme value for comprehending cycling of nutrients, ecosystem functioning, and soil health. However, comprehending such dynamics necessitate techniques capable of probing microbial interactions and metabolic activities at high spatial resolutions.

NanoSIMS, a cutting-edge analytical technique, has revolutionized the study of microbial interaction and nutrient cycling in various environments. The NanoSIMS utilization in deciphering the processes of ^{15}N assimilation by microbes represents a significant milestone in understanding the intricacies of the N cycle. Lechene et al. [117] pioneered this field by employing NanoSIMS to investigate ^{15}N uptake by *Teredinibacter turnerae*, shedding light on the N-fixation characteristics of these cells. Subsequent studies extended the scope, revealing the dynamic N flow from symbiotic nitrifiers to the wood-eating marine bivalve *Lyrodus pedicellatus* [118]. The versatility of NanoSIMS becomes evident in complex microbial communities from natural environments, where fluorescence in situ hybridization (FISH)-oriented technologies, coupled with NanoSIMS, elucidate N uptake by key players like *Chlorobium clathratiforme*, *Lamprocystis purpurea*, and *Chromatium okenii* in Lake Cadagno [119]. The ability of NanoSIMS to offer resolution at the single-cell level proves invaluable in visualization and quantification of N-assimilation [120]. Moreover, the technique captures the nuanced physiological performance of organisms like *Anabaena oscillarioides*, revealing heterogeneous assimilation of ^{15}N and ^{13}C ,

operating not only within individual cells but also extending to the larger intercellular landscapes [121]. The integration of microsensors with NanoSIMS further contributes to our understanding of significant players like *Aphanizomenon* sp. and *Nodularia spumigena* in Baltic Sea N-fluxes [122, 123].

Beyond N-cycling, NanoSIMS proves instrumental in unraveling microbial interactions, unveiling the intricacies within syntrophic and trophic connections, and the spatial dynamics in environmental microorganisms. The study of interplay among *Anabaena* and a heterotrophic epibiont, employing ^{15}N -dinitrogen and ^{13}C -bicarbonate, underscores the significance of diatom symbioses, influencing N pools and the metabolism of cyanobacterial symbionts [120, 124]. Furthermore, the real-time examination of N_2 fixation activity in Elkhorn Slough filamentous cyanobacterium-1 (ESFC-1) using NanoSIMS reveals its status as the most dynamically engaged cyanobacterial diazotroph within the mat of Elkhorn Slough [125]. The comprehensive insights provided by NanoSIMS in understanding these intricate microbial processes highlight its indispensable role in advancing our knowledge of microbial ecology and nutrient cycling. As NanoSIMS continues to be refined and applied in diverse environments, its contributions promise to deepen our comprehension of the complexities within microbial communities and their roles in nutrient transformations.

2.7. Integration of NanoSIMS with Diverse Analytical Approaches. Integrating NanoSIMS with other analytical techniques provides a powerful technique for acquiring comprehensive insights into complex geological, biological, and environmental systems. NanoSIMS, with its unparalleled spatial resolution and elemental analysis capabilities, provides detailed information about isotopic composition, elemental distribution, and molecular dynamics at the nano-scale. However, no single analytical approach can comprehend the entire complexity of such systems. Therefore, integrating NanoSIMS with complementary techniques enables researchers to leverage the strengths of each technique and overcome their individual limitations.

In the pursuit of enhancing the precision of NanoSIMS analysis, various established methods, including digital image analysis and scanning and transmission electron microscopy, have been employed for meticulous sample characterization. The aim is to discern areas ideal for a more detailed NanoSIMS analysis. These imaging techniques are seamlessly integrated with NanoSIMS, leveraging shared sample preparation requirements. Moreover, the fusion of NanoSIMS techniques with FISH has proven successful in examining microbial behavior at a sub-cellular magnitude. This approach involves the use of oligonucleotide probes equipped with distinct elemental markers, enabling the concurrent validation and characterization of specific microbes. The amalgamation of NanoSIMS and FISH brings forth numerous benefits when compared to alternative

single-cell methodologies, encompassing superior spatial precision, the capacity for multi-element or isotope examination, and quantification. Prominent combinations of NanoSIMS and FISH methodologies include improved element labeling-catalyzed reporter deposition fluorescence in situ hybridization (EL-FISH) [124], catalyzed reporter deposition-fluorescence in situ hybridization (CARD-FISH) [126, 127], and halogen in situ hybridization (HISH) [128], among others, present novel avenues for investigation. Notably, Gold-ISH, a novel non-halogen technique for phylogenetic probing, stands out as the most recent FISH-centered method complementing NanoSIMS probing [129].

Furthermore, TEM, SEM, and scanning transmission electron microscopy (STEM) are pivotal in pre-identifying microorganisms. These techniques furnish information regarding the dimensions and structure of microbiome, aiding in the investigation of cellular specimens and the precise localization of individual cells. Samples prepared for TEM imaging by embedding in paraffin or epoxy can be seamlessly repurposed for NanoSIMS investigations. Inductively coupled plasma-mass spectrometry (ICP-MS) and isotope ratio mass spectrometry (IRMS) further contribute to comprehensive isotopic bulk analysis. They are exemplified by their ability to identify alterations in marked substrates and net-fixation values, ensuring accuracy in isotopic amplifications. X-ray and STXM absorption near-edge structure play a pivotal role in mapping the distribution of OC and establishing the oxidation states of minerals or prevalent organic components. The strategic combination of X-ray analysis with NanoSIMS facilitates the mapping of essential elements for cells, along with macro-elements of physiological relevance like P, S, and silicon (Si) [130]. A noteworthy study by Remusat et al. [75] showcased the viability of NanoSIMS inquiries into distinct microstructures of soil deposited on a clear surface. The synergy of NanoSIMS and STXM-NEXAFS culminates in more resilient findings compared to standalone NanoSIMS analysis. Additionally, the microscopic observations facilitated by SEM and TEM open new avenues for investigating processes in geochemistry and ecology on a sub-micron scale.

Therefore, the strategic combination of NanoSIMS with these techniques allows for a thorough comprehension of intricate dynamics within soil ecosystems. Table 3 illustrates the utilization of different spectroscopic and imaging methods in combination with NanoSIMS to analyze soil components. The collaboration across disciplines is crucial in deciphering the complex connections existing between microbiome and their physical and environments. The concurrent examination of numerous elements through NanoSIMS has yielded valuable understandings into the spatial arrangement of elements [31, 144]. Challenges in experimental planning, sample management, and connecting nano-level observations to processes at the ecosystem scale underscore the need for extensive investigations into soil biogeochemical phenomena [75, 145], emphasizing the importance of NanoSIMS integration with other techniques in advancing soil sciences.

TABLE 3: Utilizing chosen spectroscopy and imaging methods in conjunction with NanoSIMS to analyze soil components.

Methodology	Sample specifications	Depth of analysis	Probe type	Implementations	References
Scanning electron microscope energy dispersive spectroscopy (SEM-EDS)	Pressure in the tens of torr range, with an element content exceeding 1%	Surface	Electronic	Analyzing the composition and arrangement of soil, offering details on chemical elements, and visually assessing and describing microaggregates	[131, 132]
X-ray photoelectron spectroscopy (XPS)	Ultra-high vacuum	Surface	Photoelectron	Composition of elements (dehydrogenation) in various forms of C, N, and O	[133]
Micro-X-ray fluorescence microscopy (XRF)	Flexible, micron-thin layer with element content measured in parts per million (ppm)	Variable	X-ray absorption	The makeup and chemical condition of metallic elements, with the exception of C complexes	[134, 135]
Electron probe microanalysis	High vacuum	Surface (micrometer)	Electronic	The elemental composition and dispersion, coupled with the spatial arrangement and interactions among inorganic and organic components	[136, 137]
Nuclear magnetic resonance spectroscopy	Extraction of paramagnetic compounds	—	—	Semiquantification of functional groups and recognition of components (C, N, P) Enhanced examination of elemental composition and chemical forms pertaining to C, O, and N, encompassing elemental speciation analysis and mapping	[138]
STXM	Low vacuum, helium, thin layer	Pierce through	X-ray		[139, 140]
Fourier transform infrared spectroscopy (FTIR)	Flexible, and micron-thin layer	Micrometer	Infrared light	Chemical composition of OM and minerals	[141]
Nano-scale secondary ion mass spectrometry (NanoSIMS)	High vacuum at 10^{-10} bar, smooth sample surface, with element contents measured in parts per billion (ppb) to parts per million (ppm)	Surface	Ion source	Visualizing and characterizing OM and minerals, with a particular focus on isotope mapping	[59, 72]
Infrared spectroscopy	Micrometer thin layer, frozen section	Micrometer	Infrared light	Cartographing OM distribution on aggregate surfaces and delineating the distribution of functional groups	[142]
X-ray computed microtomography	Cylinders measuring 1 mm \times 5 mm, extracted from cores with a diameter of 10 cm	Centimeter	X-ray	The findings enable a complete reconstruction of the pore network	[143]

2.8. Pros and Cons of NanoSIMS. NanoSIMS has established itself as a potent instrument in soil science, offering high spatial resolution for elemental and isotopic composition analysis. At its core, NanoSIMS functions by bombarding a sample surface with a primary beam of ions, primarily consisting of Cs^+ ions, which results in the extraction of secondary ions. These secondary ions are then analysed using a mass spectrometer, allowing for the precise determination of their mass-to-charge ratios and thus their elemental and isotopic identities. Its ability to provide detailed information at the microscale has led to valuable insights into soil properties and processes. The technique excels in visualizing microhabitats, studying microbial activities, and exploring the interactions between OM and minerals. By imaging the spatial distribution of elements such as C, N, and oxygen, NanoSIMS can provide insights into SOM dynamics, microbial activity, and nutrient cycling processes. The NanoSIMS fusion with FISH and various methodologies has expanded its application range, enabling simultaneous localization and identification of microorganisms with unique elemental tags. Moreover, NanoSIMS facilitates the analysis of multiple elements, contributing to a holistic comprehension of soil ecosystems.

Despite its strengths, NanoSIMS has notable drawbacks and limitations that necessitate careful consideration. The requirement for a minute specimen size may prove unfeasible for heterogeneous samples, and the laborious nature of the preparation process could pose challenges. The preparation of samples assumes a pivotal role in NanoSIMS analysis, particularly when dealing with intricate and diverse soil samples. This process can be both time-consuming and exacting, exerting a substantial impact on the precision of the obtained results [142]. The elevated spatial resolution might fall short for specific soil microenvironments characterized by diverse chemical compositions or structures. Isobaric interferences, occurring when two ions possess identical mass-to-charge ratios, have the potential to cause misinterpretations of data in intricate soil samples. Accurate quantification of isotopic composition faces challenges due to dead-time correction, matrix effects, and surface sensitivity. Overcoming these limitations often involves integrating NanoSIMS with complementary analytical techniques to provide a detailed and accurate specimen analysis. Overall, NanoSIMS holds potential for advancing our understanding of soil processes at the microscale, offering unprecedented information into the complex interactions between soil components and the environment. However, like any analytical technique, NanoSIMS has its pros and cons, which must be carefully considered when interpreting the outcome and designing experiments in soil science research.

3. Conclusive Remarks and Future Prospectus

NanoSIMS holds immense promise for advancing our understanding of soil ecosystems, offering avenues for groundbreaking insights. To fully unlock its potential, several key areas warrant further exploration and development.

- (1) Integrating NanoSIMS with methodologies like stable isotope probing is crucial to comprehensively unravel soil microbial functions.
- (2) Standardized procedures for both sample preparation and analysis are imperative for data reproducibility.
- (3) Investigating spatial and temporal scales is necessary for reliable conclusions on soil processes. Bridging NanoSIMS data with ecosystem function, an aspect that often lacks clarity, necessitates further exploration. Addressing these challenges has the potential to markedly improve our comprehension of soil systems.
- (4) Field applications of NanoSIMS should be explored, as evaluating its applicability and potential in real-world field conditions can transition from a predominantly laboratory-based tool to a field-ready technology.

Despite challenges in cost and technical knowhow, NanoSIMS can significantly contribute to soil science. Training, collaboration, and standardization efforts are vital for overcoming NanoSIMS-associated challenges. Future directions involve advancing imaging, refining analytics, integrating methods, and applying NanoSIMS for biogeographic and metagenomic analyses. These endeavors are expected to enhance our comprehension of soil ecology, impacting nutrient management and sustainable agriculture. The integration of molecular-scale technologies with computational modeling is poised to open new frontiers in the exploration of ecological functions within soils. Overall, the future trajectory of NanoSIMS in soil science holds significant potential for transformative contributions across diverse areas of research and practical applications.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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