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NANO-SECONDARY ION MASS SPECTROMETRY TECHNOLOGY IN THE INVESTIGATION OF SOIL ORGANO-MINERAL ASSOCIATION: A REVIEW

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ABSTRACT

Soils are a complex mixture of solid, liquid, and gaseous components, including inorganic solids like quartz, clay minerals, oxides, hydroxides, and carbonates, as well as organic matter derived from plant, animal, and microbial sources. This composition results in structural heterogeneity across various scales. During soil formation, primary particles form microaggregates, which further combine into macroaggregates, creating a hierarchical structure. Microorganisms inhabit these microhabitats, altering them as needed. Understanding of these submicron sized organo-mineral associations, the simultaneous analysis of the spatial distribution of C, N and other elements at the nano-scale allows a major step forward in the understanding of soil formation with significant implications on the soil C and N cycle, soil structural stability, nutrient cycling and the sorptive properties. Nano-Scale secondary Ion Mass Spectrometry (nano-SIMS) is a novel technology that allows for the simultaneous analysis of up to seven ion species with high sensitivity and resolution, down to 50 nm. The objective of this seminar is to understand this advanced tool that offers unprecedented insights into the elemental and isotopic composition of soils at the submicron scale.

Keywords : Organo-mineral carbon association, reactive minerals, Nano-Scale secondary Ion Mass Spectrometry (nano-SIMS), sputtering, oxidative decomposition

Introduction

Organic matter must decay to release the energy and nutrients that fuel all life processes on Earth. (Janzen, 2006) Consequently, the processes and mechanisms that retain organic matter (OM) in soil are of central importance to various branches of environmental research.

For instance, an agronomist might aim to align periods of high nutrient and energy release with the growth stages of crops. An environmental chemist may seek to either immobilize an organic soil contaminant or accelerate its breakdown into less harmful substances. Meanwhile, climate scientists need to understand the processes that regulate the production of potent greenhouse gases from decomposing OM. Associations of organic matter (OM) with pedogenic minerals, referred to as mineral-organic associations (OMAs), are recognized as crucial factors influencing these and many other processes. (Kleber *et al.*, 2015).

More recently, the role of soil minerals in protecting OM has been recognized as a key mechanism for long-term carbon storage. Most

organic carbon in soil is found in close proximity to minerals such as phyllosilicates, aluminosilicates, metal oxides, hydroxides, oxyhydroxides, carbonates, and sulfides, and it will almost inevitably come into contact with these mineral surfaces during its cycle.

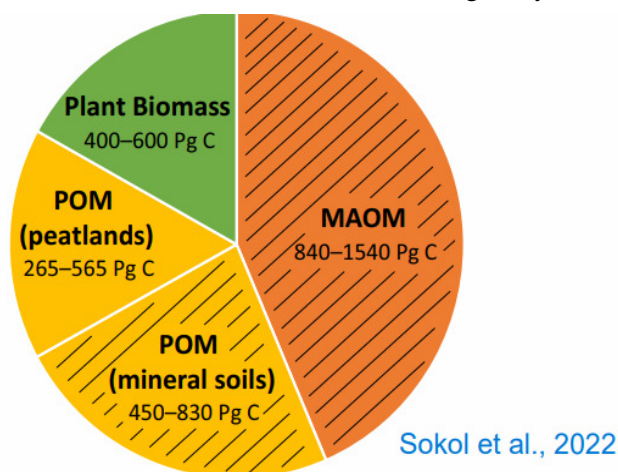


Fig. 1: Earth's major terrestrial pools of organic carbon. (Sokol *et al.*, 2022)

As shown in the figure 1, with in mineral soils (shown in dashed lines), approximately 65% of soil organic carbon (SOC) is mineral-associated organic matter carbon (MAOM-C), whereas ~35% of SOC is particulate organic matter carbon (POM-C). MAOM-C represents ~34%–51% of total terrestrial organic carbon globally. Novel techniques are available for in situ characterization and correlation between soil organic matter (SOM) and highly reactive minerals at submicron scale. One of which is nano-SIMS.

AN OVERVIEW OF ORGANO-MINERAL ASSOCIATION

The association of organic matter (OM) with pedogenic minerals is referred to as mineral–organic associations (MOAs) or organo-mineral associations (OMAs) (Kleber *et al.*, 2015). In soils, these associations are primarily studied for their role in determining the long-term retention of OM. Soil organic matter (SOM) is defined as "the sum of all naturally derived organic materials present." (Baldock and Broos, 2011). However, not all organic materials, such as roots, fauna, and plant debris, equally contribute to the formation of OMAs, making it essential to identify the reactive and mobile forms of SOM. Oxidative depolymerization adds ionizable, oxygen-containing functional groups to decomposition products, increasing their aqueous solubility and reducing their molecular size as microbial decomposition advances. According to the biodegradation/sorption model (Hedges and Keil,

1999), some degradation intermediates evade microbial uptake and leach into the soil, where they partition between the aqueous phase and the surrounding soil matrix. Although the degree of organic fragment functionalization varies with soil ecosystem, type, and horizon, it is clear that the functionality of decomposition products always depends on the presence and composition of the aqueous phase, the soil solution.

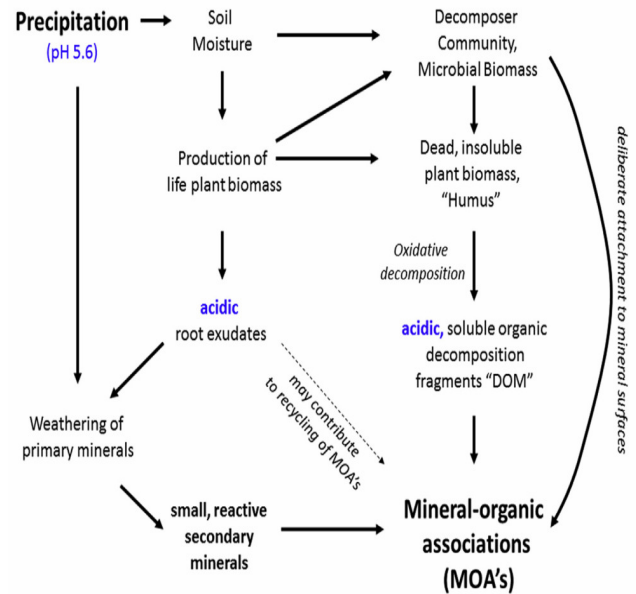


Fig. 2: The causal relationship between moisture supply, plant biomass production, mineral weathering, and the formation of mineral–organic associations. (Kleber *et al.*, 2015)

MINERALS IN SOIL ORGANO-MINERAL ASSOCIATION

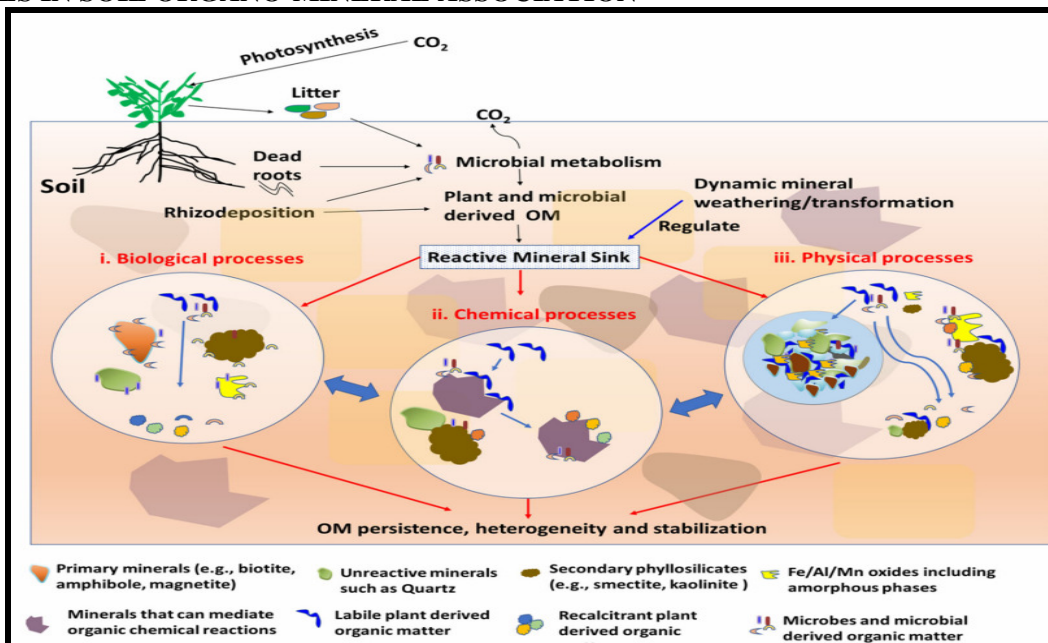


Fig 3: Diagram showing the “Reactive Mineral Sink” (RMS)-driven organic matter processing, resynthesis, transformation, transport and stabilization in soils. (Wu *et al.*, 2023)

The Reactive Mineral System (RMS) can be described as a matrix consisting of various minerals that are distinguished by a high density of surface charges and/or substantial thermodynamic potential. This matrix includes both less stable primary minerals, such as biotite and amphibole, as well as relatively stable secondary minerals like smectite, montmorillonite, vermiculite, and kaolinite, along with amorphous minerals like Fe (III)/Mn (IV)/Al (III) oxyhydroxides. (Wu *et al.*, 2023)

Reactive minerals are crucial in regulating the dynamics of organic matter (OM) in soil through

physical, chemical, and biological processes. In physical processes, mineral coagulation and dispersion play a key role in aggregate formation and turnover, which in turn affects soil structure and porosity, leading to the protection or liberation of organic matter. The mineral matrix also influences hydrological processes that control the movement of organic matter, microbes, colloids, and solutes through the matrix. This results in the relocation, reprocessing, and stabilization of organic matter. (Wu *et al.*, 2023).

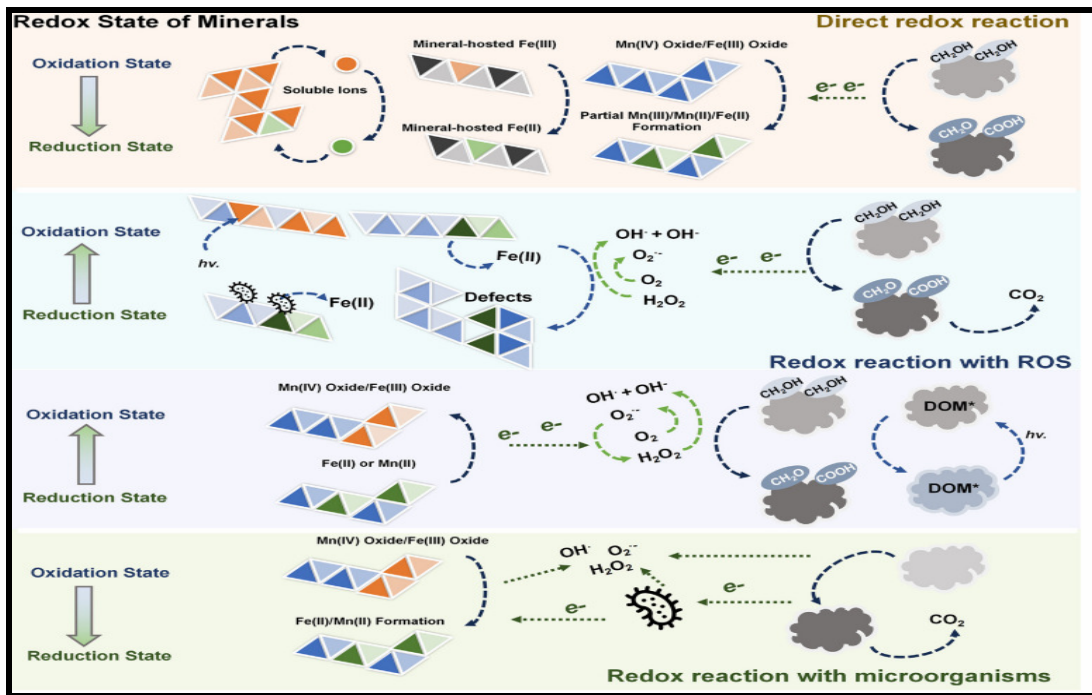


Fig. 4 : Redox reactions between organic carbon and minerals with the support of reactive oxygen species (ROS) and microorganisms. (Xu & Tsang, 2024)

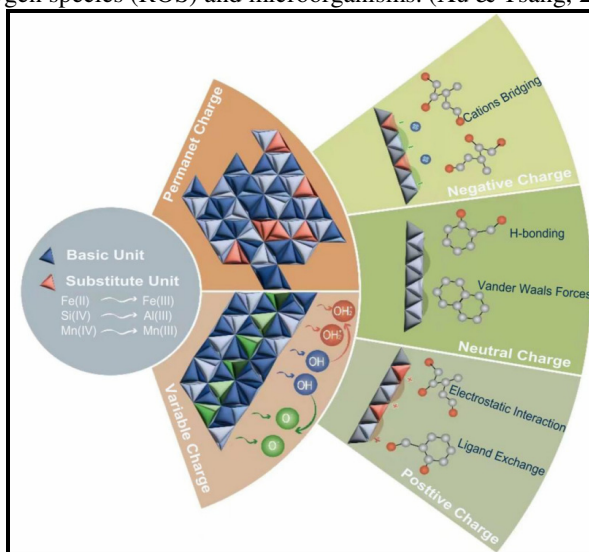


Fig. 5: Sorption mechanisms of soil organic carbon on minerals with different properties. (Xu & Tsang, 2024)

In biological processes reactive minerals can directly bind to exoenzymes (Mahmoudi *et al.*, 2023) impacting the enzymatic decomposition and transformation of organic matter. Additionally, the mineral matrix can form micron-sized niches that support diverse microbial communities, which can further influence the decomposition, reprocessing, and resynthesis of organic matter. (Hartmann and Six, 2022; Kravchenko and Guber, 2017)

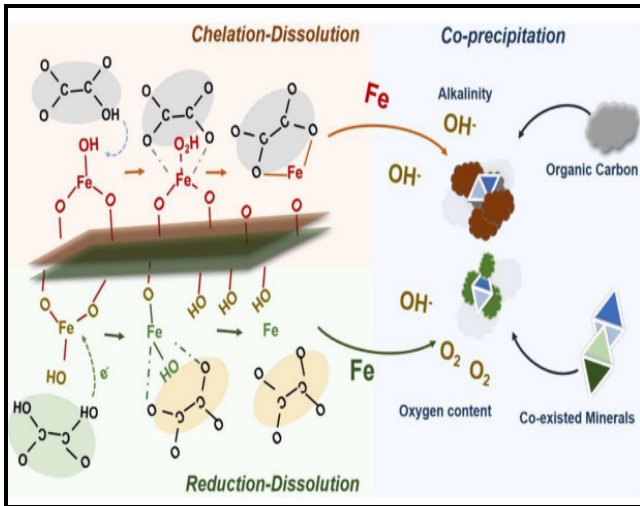


Fig. 6: Dissolution of minerals with organic carbon through chelation or reduction; subsequent co-precipitation of soluble metal ions with organic carbon and other minerals. (Xu & Tsang, 2024)

In chemical processes, various mineral surfaces exhibit distinct chemical reactivities, leading the reactive mineral matrix to create a heterogeneous distribution of organic matter and drive molecular transformation. This occurs through mechanisms such as the adsorption of organic ligands onto mineral surfaces, intercalation, cation bridging, co-precipitation with minerals, and the subsequent formation of organo-mineral associations.

Analytical approaches to study organo-mineral association.

Bulk analysis techniques for studying organo-mineral associations (OMAs) include:

- Thermogravimetric (TG) analysis combined with differential scanning calorimetry (DSC)
- FTIR spectroscopy
- Stable isotope tracer techniques

These methods reveal information about OMAs by examining pH, ionic strength, points of zero charge, zeta-potential, surface site density, and specific surface area (SSA). For detailed, small-scale soil phenomena, advanced instrumental techniques are crucial. In situ

analysis of undisturbed soil samples is preferred over fractionation methods. Imaging techniques, including scanning electron microscopy (SEM), transmission electron microscopy (TEM), and synchrotron-based methods (e.g., STXM, NEXAFS), are essential for understanding spatial relationships and structural details in OMAs. (Kleber *et al.*, 2015)

AN OVERVIEW OF NANO-SIMS

What is the basic principle of Secondary Ion Mass Spectrometry (SIMS)?

Secondary Ion Mass Spectrometry (SIMS) is based on sputtering of a few atomic layers from the sample surface by a primary ion beam and analyzing the emitted neutral and charged particle called secondary ions ejected from the sample with a mass spectrometer, distinguished by their mass-to-charge ratio. This technique is one of the most sensitive elemental and isotopic surface analysis techniques. (Becker and Dietze, 2000)

SIMS instrument

SIMS (Secondary Ion Mass Spectrometry) instruments are analytical tools used to analyze the surface composition of materials at the atomic or molecular level.

How SIMS Works

1. Primary Ion Beam: A focused beam of primary ions (like O_2^+ , Cs^+ , or Ga^+) is directed at the surface of the sample.
2. Sputtering: The impact of the primary ions causes atoms and molecules from the sample surface to be ejected (sputtered) as secondary ions. These secondary ions can be mono- or polyatomic. These polyatomic ions are mainly the result of the recombination of several monoatomic particles occurring close to each other at the sample surface. (McMahon *et al.* 2006)
3. Mass Spectrometry: These secondary ions are collected and After sorting in energy in an electrostatic sector of a mass spectrometer, they become separated in a magnetic field by their mass/charge ratio, and pre-selected masses can finally be analysed (Guerquin-Kern *et al.*, 2005).

What is nano SIMS?

Nano-scale secondary ion mass spectrometry (Nano-SIMS) belongs to the latest generation of SIMS instruments. It allows for the simultaneous analysis of up to seven secondary ion species (Nano-SIMS 50L) with high sensitivity and high lateral resolution at a submicron scale. The spatial information remains

preserved in such a way that the distribution of the various masses can be visualized. (Guerquin-Kern *et al.*, 2005)

What is the advantage of nano-SIMS over previous SIMS generations:

In comparison to previous SIMS generations, the high mass resolution at high transmission allows the differentiation between masses with differences of less than fractions of 1 atomic mass unit (u), for instance between $^{13}\text{C}^{14}\text{N}^-$ (27.016 u) and $^{12}\text{C}^{15}\text{N}^-$ (27.009 u). (Guerquin-Kern *et al.* 2005; Lechene *et al.* 2006; Boxer *et al.* 2009). Consequently, the Nano-SIMS is capable of analysing both the elemental and the isotopic composition of a solid sample at submicron scale. This allows in understanding of soil formation, soil structural stability, and adsorption properties of soil interface.

Nano-sims technology in the investigation of organo-mineral association

Primary and secondary beams:

Cesium (Cs^+) or oxygen (O^-) primary ions are used, which enhance the ionization yield of the secondary ions significantly compared to nonreactive primary ions (Boxer *et al.*, 2009) Because of the coaxial optical design of the primary and secondary ion optics, only secondary ions of opposite charge of the primary ions can be analyzed.

Accordingly, with Cs^+ primary ions, negatively charged secondary ions, e.g., $^{12}\text{C}^-$, $^{13}\text{C}^-$, $^{12}\text{C}^{14}\text{N}^-$, $^{12}\text{C}^{15}\text{N}^-$, and $^{28}\text{Si}^-$, can be detected with a lateral resolution down to 50 nm, whereas with O^- primary ions, positively charged secondary ions such as $^{23}\text{Na}^+$, $^{28}\text{Si}^+$, $^{39}\text{K}^+$, and $^{40}\text{Ca}^+$ can be collected at a lateral resolution down to 150 nm.

The primary ion beam intensity is quite low (about 0.1 pA), which makes it hard to detect elements with low secondary ion yields. Therefore, the primary ion current during imaging varies between ~0.8 and ~1.4pA (Heister *et al.*, 2012; Remusat *et al.*, 2017; Rennert *et al.*, 2014; Xiao *et al.*, 2015; Yu & Liu, 2022)

Sample preparation - a crucial prerequisite for meaningful Nano-SIMS measurements.

Soil colloids are extracted by suspending air-dried soil in deionized water (1:5 W/V), shaking for 8 hours at 25°C, and centrifuging for 6 minutes at 2,500 g. The supernatant is stored in 50 mL vials at 4°C and analysed within a week. (Schumacher *et al.*, 2005). The sample are coated with gold using physical vapor deposition under an Ar atmosphere to counteract the

insulating properties of the soil and resin. Additional charging issues were managed with an electron flood gun when using the Cs^+ source. While these methods were effective, other techniques, such as deposition on filters (Musat *et al.* 2008) or embedding in elemental sulfur (Lehmann *et al.* 2008) are also possible. Prior to analysis, the coating layer and possible contamination of the sample surface are sputtered away by using a high primary beam current between 500 and 600 pA. (Heister *et al.*, 2012; Remusat *et al.*, 2017; Rennert *et al.*, 2014; Xiao *et al.*, 2015; Yu & Liu, 2022)

Pure biological or geological samples v/s soil samples:

Unlike pure biological or geological samples, soil samples are complex because they contain various minerals, organic matter (OM), and microorganisms. During soil formation, these inorganic and organic components interact closely and form aggregated structures, resulting in materials that are structurally heterogeneous across different temporal and spatial scales. This complexity makes sample preparation challenging and identification of the various components difficult (Herrmann *et al.*, 2007; Totsche *et al.*, 2010). However, recent studies demonstrated that Nano-SIMS can effectively differentiate between organic and inorganic components and identify minerals within these complex soil samples. (Heister *et al.*, 2012)

The most promising nano-SIMS instrumental setup for soil related application:

- According to Senoner and Unger (2012), The best setup for soil-related applications in Nano-SIMS involves using a Cs^+ primary ion beam and collecting negatively charged secondary ions like $^{12}\text{C}^-$, $^{16}\text{O}^-$, $^{12}\text{C}^{14}\text{N}^-$, and $^{28}\text{Si}^-$. This is because the yield of negatively charged ions such as $^{12}\text{C}^-$ and $^{28}\text{Si}^-$ is much higher than their positively charged counterparts ($^{12}\text{C}^+$ and $^{28}\text{Si}^+$). For example, nitrogen (N) can only be detected as a negatively charged ion ($^{12}\text{C}^{14}\text{N}^-$). However, elements that are detected only as positively charged ions, like $^{27}\text{Al}^+$ and $^{56}\text{Fe}^+$, cannot be directly analyzed in this mode. Instead, they can be detected as their partial oxides ($^{27}\text{Al}^{16}\text{O}^-$ and $^{56}\text{Fe}^{16}\text{O}^-$). The tendency of an element to ionize as either an anion or cation depends on its electronic configuration. Therefore, some elements like Na, K, and Ca are better measured using an O^- primary ion beam.
- For the Nano-SIMS measurements, an aliquot of the colloidal suspension is dropped onto a silicon wafer (Xiao *et al.*, 2015) or onto a gold foil (Yu & Liu, 2022) or mirror-polished metal stubs (Heister

et al., 2012) or onto clean Si₃N₄ windows without the use of any fixative (Remusat *et al.*, 2017) as sample holders & the analysis is performed with a Nano-SIMS 50L (Cameca, Gennevilliers, France)

- Secondary ion images of $^{12}\text{C}^-$, $^{12}\text{C}^{14}\text{N}^-$, $^{27}\text{Al}^{16}\text{O}^-$, and $^{56}\text{Fe}^{16}\text{O}^-$ are simultaneously collected by electron multipliers with an electronic dead time of 44 ns. The estimated depth resolution when using 16 keV Cs⁺ ions for the primary ion beam is approximately 15 nm. All the measurements were performed in the imaging mode, with a dwell time of 1 ms pixel⁻¹ for all the acquisitions. (Heister *et al.*, 2012; Remusat *et al.*, 2017; Rennert *et al.*, 2014; Xiao *et al.*, 2015; Yu & Liu, 2022)
- The presence of $^{12}\text{C}^-$ and $^{12}\text{C}^{14}\text{N}^-$ ions is an indicator of C and organic matters from soil, respectively. $^{27}\text{Al}^{16}\text{O}^-$ ions can be attributed to aluminum minerals or oxides, while $^{56}\text{Fe}^{16}\text{O}^-$ ions belongs to iron minerals or oxides. ROIs (numbered regions of interest) were chosen according to the distribution of the secondary ions of $^{12}\text{C}^-$ and $^{12}\text{C}^{14}\text{N}^-$ using Image J (1.45 h version) with the Open-MIMS plugin. Areas and sums of secondary ion counts for each ROI are extracted from all images. All ROIs with an area greater than 20 pixels were used for further calculations. The triangle algorithm is used for this measurement. (Heister *et al.*, 2012; Remusat *et al.*, 2017; Rennert *et al.*, 2014; Xiao *et al.*, 2015; Yu & Liu, 2022)

Characterization of OMAs by microscopy (light, SEM) prior to nano-SIMS analysis:

In order to obtain an overview of the samples and to find relevant regions of interest for Nano-SIMS analysis, the samples are first inspected by reflected light microscopy and scanning electron microscopy in combination with energy dispersive X-ray spectroscopy (SEM-EDX) for elemental analysis on the micro-meter scale. Then the Nano-SIMS analyses are performed with a Nano-SIMS 50 L (Cameca, Gennevilliers, France).

Electron-based imaging approaches such as scanning electron microscopy and TEM are very helpful for acquiring images of the physical structure and arrangement OM with minerals, but they are not chemically sensitive to the nature of the OM or mineral (although something can be learned about the mineral phase either from elemental composition via electron dispersive X-ray spectroscopy or by measuring d-spacings between mineral lattices in high-resolution TEM).

Differentiation between organic matter & minerals in OMAs:

The presence of $^{12}\text{C}^-$ and $^{12}\text{C}^{14}\text{N}^-$ ions is an indicator of C and organic matters from soil, respectively. $^{27}\text{Al}^{16}\text{O}^-$ ions can be attributed to aluminum minerals or oxides, while $^{56}\text{Fe}^{16}\text{O}^-$ ions belongs to iron minerals or oxides.

Fig 7 indicated that All minerals had high $^{16}\text{O}^-$ rates and were identified as illite due to their high $^{28}\text{Si}^-$ and $^{27}\text{Al}^{16}\text{O}^-$ signals. Small areas with $^{56}\text{Fe}^{16}\text{O}^-$ signals indicate the presence of ferrihydrite, although these signals might also come from iron in illite. Normally, ferrihydrite can be distinguished from illite by the absence of $^{27}\text{Al}^{16}\text{O}^-$, but this distinction is difficult when small ferrihydrite particles are on illite. Comparing the $^{56}\text{Fe}^{16}\text{O}^-$ amounts from illite and ferrihydrite is not possible due to different mineral matrices, which affect measurement accuracy in SIMS and Nano-SIMS analyses. Standard materials for accurate comparison are unavailable because of soil heterogeneity.

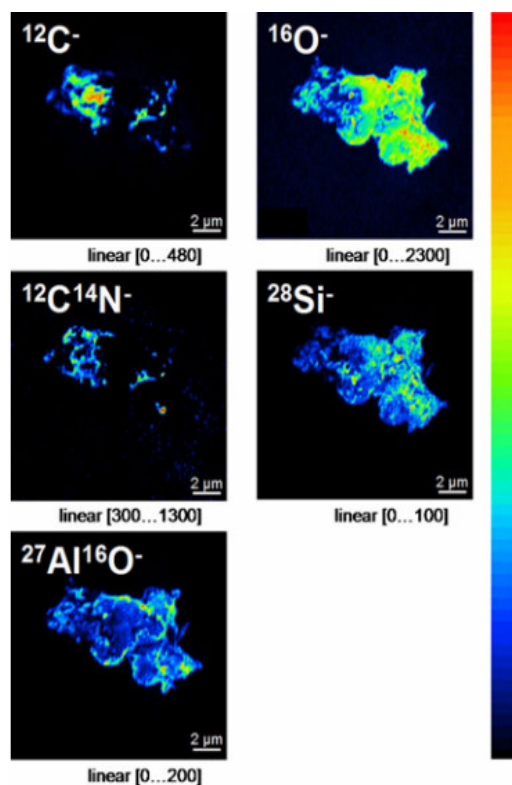


Fig. 7: Nano-SIMS images (256×256 pixels) of the $^{12}\text{C}^-$, $^{16}\text{O}^-$, $^{12}\text{C}^{14}\text{N}^-$, $^{28}\text{Si}^-$, $^{27}\text{Al}^{16}\text{O}^-$, and $^{56}\text{Fe}^{16}\text{O}^-$ distribution acquired on the particles (30×30 μm) of an agglomeration having size fraction of < 20 μm of artificial soil containing ferrihydrite & illite along with quartz & manure. The hue scale indicates the counts per second and pixel of the secondary ions. (Heister *et al.*, 2012)

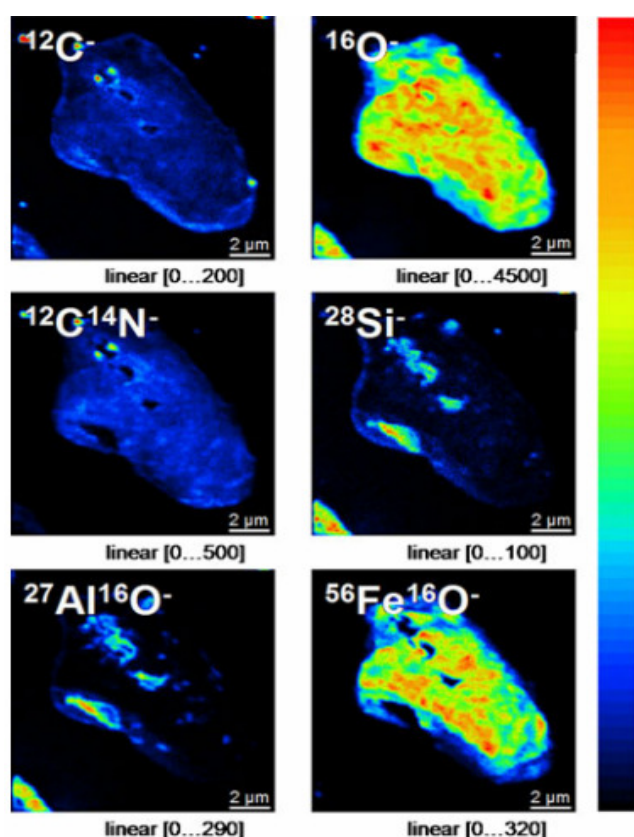


Fig. 8: Nano-SIMS images (256×256 pixels) of the $^{12}\text{C}^-$, $^{16}\text{O}^-$, $^{12}\text{C}^{14}\text{N}^-$, $^{28}\text{Si}^-$, $^{27}\text{Al}^{16}\text{O}^-$, and $^{56}\text{Fe}^{16}\text{O}^-$ distribution acquired on the ferrihydrite particle in the particle size fraction <20 μm of artificial soil containing illite & ferrihydrite along with manure & quartz. (Heister *et al.*, 2012)

Fig. 8 showed that the ferrihydrite was identified by high $^{16}\text{O}^-$ and $^{56}\text{Fe}^{16}\text{O}^-$ yield & the $^{12}\text{C}^-$ signal on ferrihydrite was much lower and more evenly distributed compared to illite shown in figure 7, indicating organic material was present but distributed differently on ferrihydrite.

Heister *et al.* (2012) investigated the density fraction of 1.8–2.4 g cm^{-3} . It was assumed that the organo-mineral associations were found in this fraction, as the free organic material will be lighter than 1.8 g cm^{-3} and the minerals without organic material attached mostly heavier than 2.4 g cm^{-3} . The particles were recognized by SEM-EDX as illite with attached organic constituents. The aggregates shown in Figs. 9 was recognized as illite due to their high rates of $^{16}\text{O}^-$, $^{28}\text{Si}^-$, and $^{27}\text{Al}^{16}\text{O}^-$. In addition, $^{12}\text{C}^-$ and $^{12}\text{C}^{14}\text{N}^-$ secondary ions were detected either in a network-type structure on the clay platelets or at the

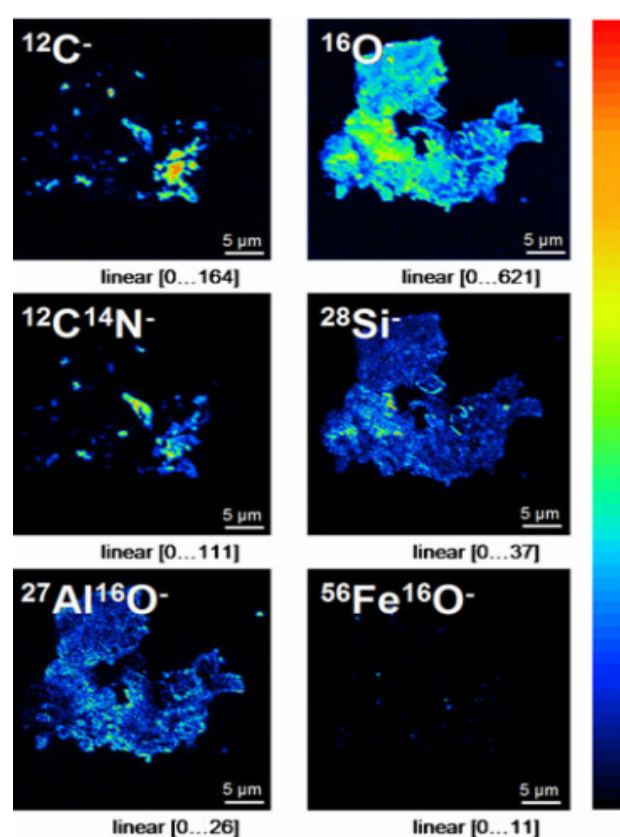


Fig. 9 : Nano-SIMS images (256×256 pixels) of the $^{12}\text{C}^-$, $^{16}\text{O}^-$, $^{12}\text{C}^{14}\text{N}^-$, $^{28}\text{Si}^-$, and $^{27}\text{Al}^{16}\text{O}^-$ distribution acquired on an aggregate of the density fraction 1.8–2.4 g cm^{-3} of the artificial soil containing illite and boehmite along with manure and charcoal. (Heister *et al.*, 2012)

rim. From the result of fig 6-8, Heister *et al.* (2012) concluded that the organic material attaches to the clay minerals and forms a coating on their surface & the organic material attaches in small, discrete patches on dedicated sites of the clay minerals.

Similarly, Remusat *et al.* (2017) (Fig. 10) found heterogeneous distribution of organic matter as a very thin coating or as patches on a soil microaggregate in subsurface layers, possibly in protective pores. Nitrogen and carbon locations differ significantly, indicating a variable Organic matter composition. Carbon was distributed in patches all over the particle, while N is found in some patches and forms a discontinuous rim around the particle.

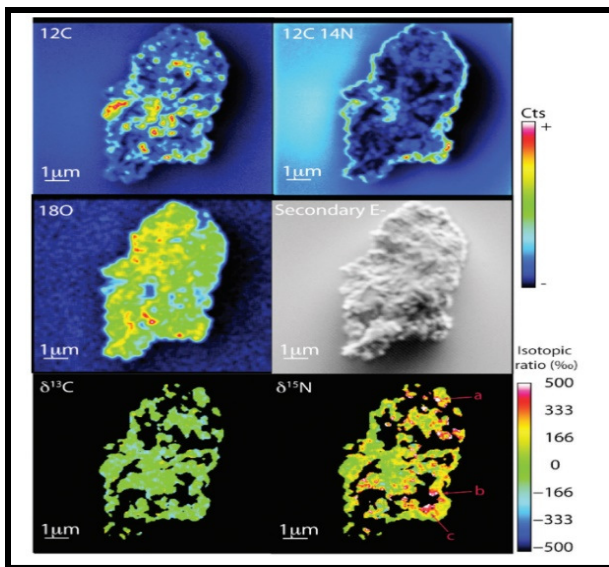


Fig. 10: Nano-SIMS images of a soil microaggregate (density :1.8–2.0 g cm⁻³, C/N = 16, $\delta^{13}\text{C} = -27\%$ relative to PDB, 210 mg C/g of fraction), collected from forest topsoil located at Ebrach, Germany where ^{15}N labeled leaves were deposited 12 years before sampling. [Remusat *et al.* (2017)]

Yu & Liu (2022) (Fig 11) found heterogeneous distribution of soil colloids on golden foil from SEM image of soil water dispersible colloids from treatments: (1) without fertilization (Control), (2) farmyard manure fertilization (M), and (3) inorganic fertilization as nitrogen, phosphorus, and potassium fertilizer combination (NPK) from a LTFE 1986–2018) from the Institute of Red Soil, Jinxian Country, excluding topographic effects. To investigate whether Al and Fe minerals in soil colloids preserve soluble C and N, Nano-SIMS was used to observe their associations. SEM images showed heterogeneous distribution of soil colloids on golden foil, excluding topographic effects. Nano-SIMS images revealed $^{12}\text{C}^-$ and $^{12}\text{C}^{14}\text{N}^-$ closely associated with $^{27}\text{Al}^{16}\text{O}^-$ and $^{56}\text{Fe}^{16}\text{O}^-$ in manure-fertilized soil. They found [Fig 11 (B)] 25–75% of the $^{27}\text{Al}^{16}\text{O}^- / ^{12}\text{C}^-$ and $^{27}\text{Al}^{16}\text{O}^- / ^{12}\text{C}^{14}\text{N}^-$ ratios were in the following order: M > Control > NPK. In contrast, the 25–75% of the $^{56}\text{Fe}^{16}\text{O}^- / ^{12}\text{C}^-$ and $^{56}\text{Fe}^{16}\text{O}^- / ^{12}\text{C}^{14}\text{N}^-$ ratios were in the following order: NPK > M > Control.

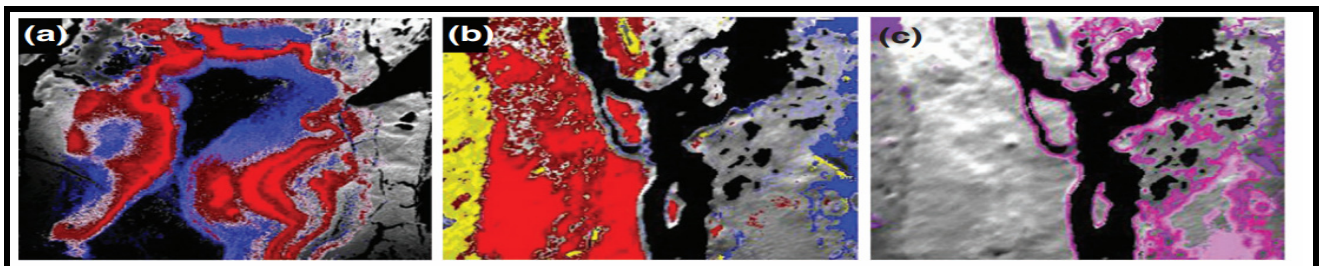


Fig. 12: Nano-SIMS measurements on a thin section of a nodule from a Bg horizon of a Stagnosol. Areas representing Fe (red), Mn (blue), silicates (yellow), the transition fringe (pink/purple) and resin (black). Rennert *et al.* (2014).

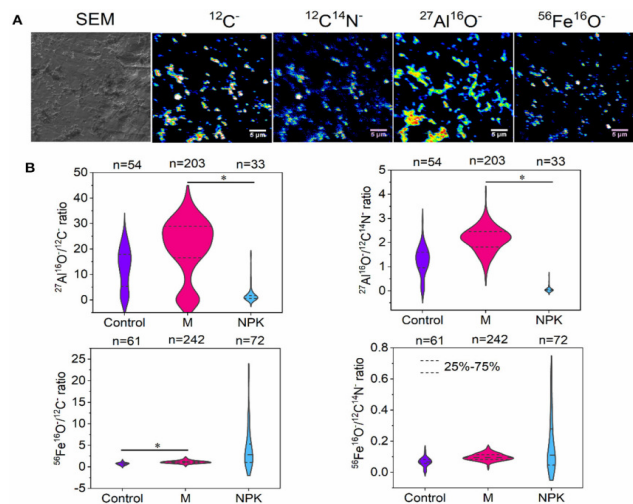


Fig. 11: (A) Typical scanning electron microscope (SEM) and nano-scale secondary ion mass spectrometry (Nano-SIMS) images of a soil water dispersible colloid collected from a long-term field experiment (1986–2018) from the Institute of Red Soil, Jinxian Country where treatments were (1) without fertilization (Control), (2) farmyard manure fertilization (M), and (3) inorganic fertilization as nitrogen, phosphorus, and potassium fertilizer combination (NPK).

(B) Violin charts of the ratios of minerals and organics based on the region of interest (ROI) analysis. [Yu & Liu (2022)]

Yu & Liu (2022) concluded that long-term manure fertilization increased Al mineral association with organic matter. Both organic and inorganic fertilization treatments increased Fe mineral association with organic matter, promoting organo-mineral associations due to the fact that higher short-range ordered Fe minerals like proto-imogolite, allophane, ferrihydrite, and hisingerite have a strong adsorption on soil organic matter.

Rennert *et al.* (2014) studied a 12 mm nodule from the Bg horizon of a *Stagnosol* in Eastern Germany by embedding it in epoxy resin and polishing it to a thin section of 32 μm with a goal to explore the spatial distribution of soil organic matter (SOM) and potential sorbents using Nano-SIMS for sub-micrometer-scale resolution.

Rennert *et al.* (2014) used SEM-EDS and nano-SIMS to examine Fe and Mn oxides in a nodule, revealing complex interactions during redox cycles. They found that Fe oxide zones were highly pure with minimal organic matter (OM), suggesting redox-cyclic formation. Mn accumulation zones, adjacent to transition areas, showed higher SOM, indicating stronger OM association in these regions.

Similar results were found by Xiao *et al.* (2015) in their study of long-term agricultural soil colloids at submicron scale. Fig 13 shows the Nano-SIMS

derived high-resolution spatial distribution images (100–200 nm) for various ions. The $^{12}\text{C}^-$ and $^{12}\text{C}^{14}\text{N}^-$ images indicated the presence of soil organic carbon (SOC) and soil organic nitrogen (SON). $^{27}\text{Al}^{16}\text{O}^-$ images pointed to Al minerals like allophane and imogolite, $^{56}\text{Fe}^{16}\text{O}^-$ images showed Fe minerals such as ferrihydrite. The intensity variation (from blue to white) in Nano-SIMS images demonstrated the heterogeneous distribution of mineral-organic associations (MOAs) at the submicron scale.

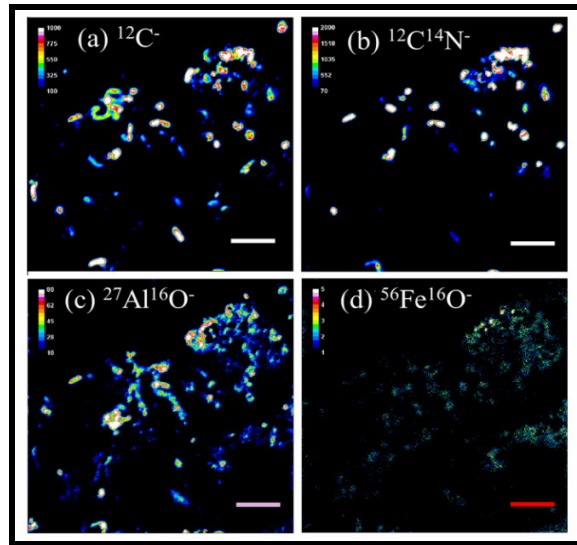


Fig. 13: Nano-SIMS images of the (a) $^{12}\text{C}^-$ (b) $^{12}\text{C}^{14}\text{N}^-$, (c) $^{27}\text{Al}^{16}\text{O}^-$ and (d) $^{56}\text{Fe}^{16}\text{O}^-$ distribution of the soil organo-mineral association surface (30 X 30 μm) with a 512 X 512pixel resolution on a soil colloid obtained from a MNPK (swine manure plus chemical nitrogen, phosphorus and potassium) treatment site (1990–2013) conducted by the Chinese Academy of Agricultural Sciences, Qiyang, China. (Xiao *et al.*, 2015)

Xiao *et al.* (2015) from their Correlation analysis (see figure: 14) showed a significant relationship between SOC ($^{12}\text{C}^-$) and Al minerals $^{27}\text{Al}^{16}\text{O}^-$, $R^2 = 0.75$, but a weaker correlation with Fe minerals

($^{56}\text{Fe}^{16}\text{O}^-$), with $R^2 = 0.37$. Similarly, SON ($^{12}\text{C}^{14}\text{N}^-$) was significantly correlated with Al minerals ($^{27}\text{Al}^{16}\text{O}^-$) { $R^2 = 0.56$ } but not with Fe minerals ($^{56}\text{Fe}^{16}\text{O}^-$) { $R^2 = 0.11$ }.

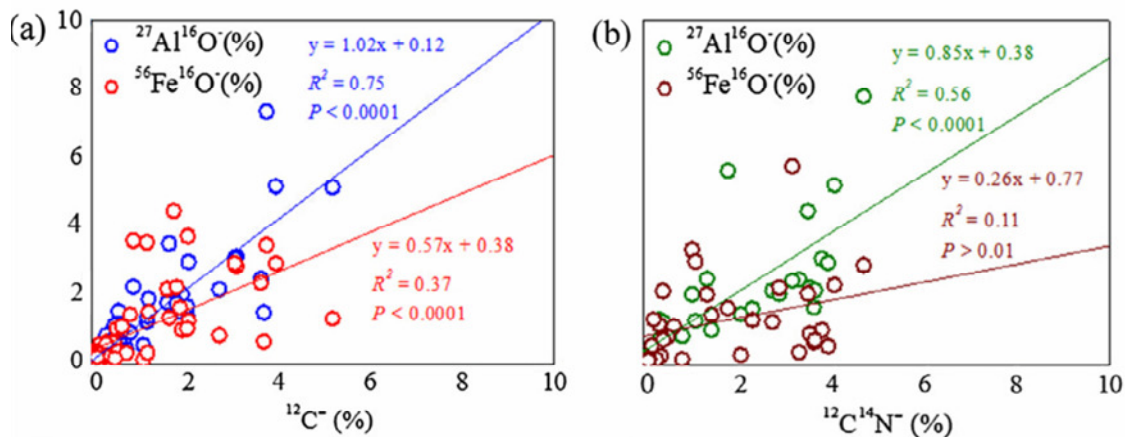


Fig 14: (a) And (b) Scatter plot of the percentages of $^{12}\text{C}^-$, $^{12}\text{C}^{14}\text{N}^-$, $^{27}\text{Al}^{16}\text{O}^-$ and $^{56}\text{Fe}^{16}\text{O}^-$ generated from each of the numbered regions of interest (ROIs) areas shown in Fig. 13. [Xiao *et al.* 2015]

Xiao *et al.* (2015) reasoned more significant correlation of SOM with the Al minerals than with the Fe minerals as the long-term organic inputs preserved the beneficial microbial community and delayed soil acidification, which favoured the formation of Al minerals binding strongly with SOM. However, high proton concentrations could alter the soil Fe oxidation state by promoting the conversion of Fe (III) to Fe (II), thus causing a lower affinity for organic matter, which might explain the less significant correlation relationship between SOM and $^{56}\text{Fe}^{16}\text{O}^-$ than that between SOM and $^{27}\text{Al}^{16}\text{O}^-$.

Limitations of nano-sims in investigation of soil organo no mineral association

Scanning X-ray Transmission Microscopy in combination with Near Edge X-ray Absorption Fine Structure spectroscopy & Nano-SIMS operate at similar scales, they theoretically provide complementary information about organic matter in soil microstructures. But Nano-SIMS is a surface technique probing only a few tens of nm deep whereas

STXM is a transmission technique averaging information over the sample's entire thickness. The major discrepancy is organic matter on the sample's back is invisible to Nano-SIMS but detected by STXM.

This finding was shown by Remusat *et al.* (2017) in their Nano-SIMS Study of Organic Matter Associated with Soil Aggregates, combined with STXM technology (See Fig 14). They found that Nano-SIMS Showed organic matter (OM) as a thick coating on one side of the particle whereas STXM detected OM over a larger area of the sample. This illustrates that Nano-SIMS and STXM do not image the sample in the same way. Only areas with concomitant Nano-SIMS and STXM signals (like areas 1, 2 & 3 in figure A, B & C) were taken into account for joined interpretations. The C-NEXAFS spectrum extracted for these areas revealed a broad and strong absorption band around 285.2 eV produced by aromatic C and a second distinct peak at 288.5 eV in the carboxylic region.

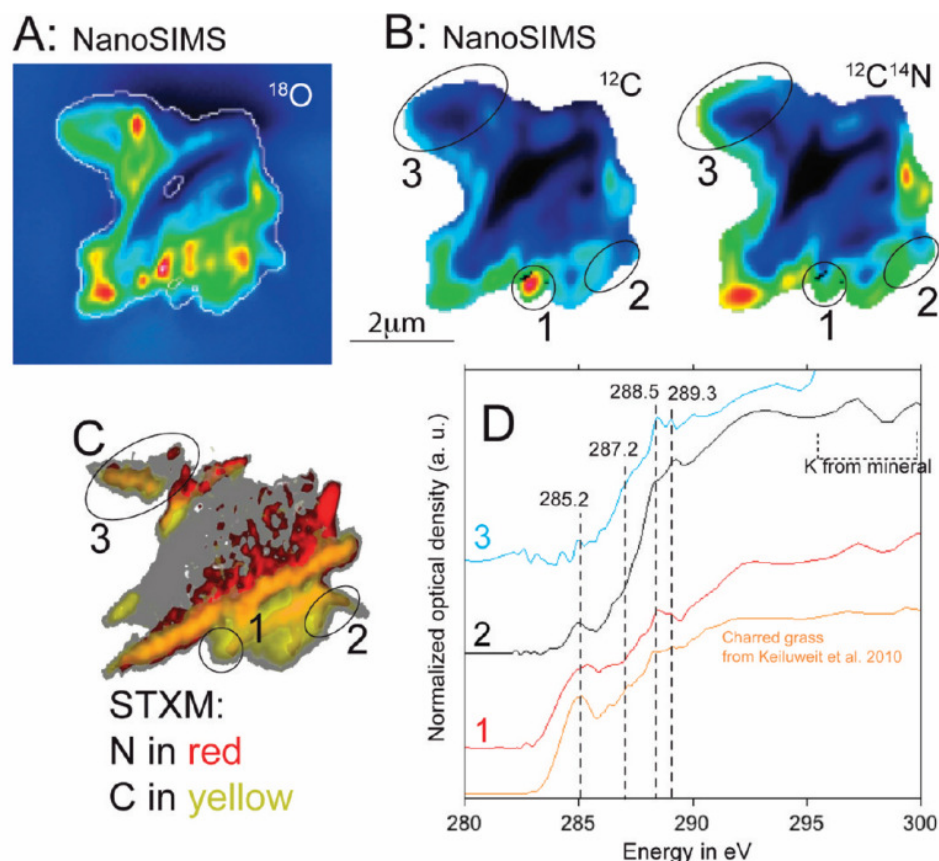


Fig. 15: The combined study of a soil aggregate (5µm wide) by Nano-SIMS (A and B) and STXM/NEXAFS (C and D) collected from forest top layer in Ebrach, Germany where, Germany where ^{15}N labeled leaves were deposited 12 years before sampling. (Remusat *et al.*, 2017)

Combined use of nano-sims with other imaging technologies in investigation of soil omas

Imaging helps to determine the spatial relationships of the various components of OM mineral associations, and more than many other, it helps us to build conceptual models of their formation by appealing to our visual senses. However, it is important to remember that particular techniques are only sensitive to a particular type of phenomenon and therefore there are no images that may provide a fully comprehensive picture of MOAs.

Electron-based imaging approaches such as scanning electron microscopy and TEM are very helpful for acquiring images of the physical structure and arrangement OM with minerals, but they are not chemically sensitive to the nature of the OM or mineral (although something can be learned about the mineral phase either from elemental composition via electron dispersive X-ray spectroscopy or by measuring d-spacings between mineral lattices in high-resolution TEM).

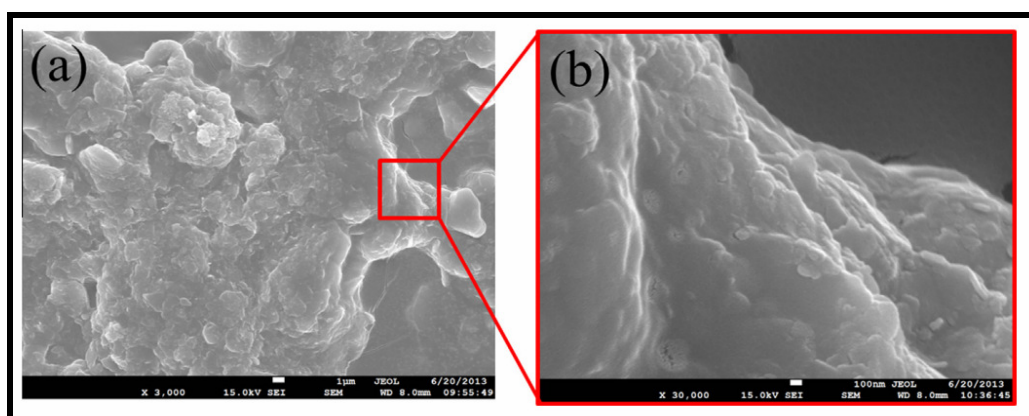


Fig. 16: The SEM images of the organo-mineral association surface. (a) The SEM image of the organo-mineral association surface at the micron scale, bar = 1 μm . (b) Superimposed SEM image of the organo-mineral association surface at the nano-scale. Bar = 100 nm. (Xiago *et al.*, 2015)

Xiago *et al.*, 2015 in their in-situ visualization and characterization study of highly reactive minerals to preserve soil organic matter (SOM) in colloids showed the SEM images of the soil organo-mineral association which revealed rough and clustered surfaces of soil organo-mineral associations at both micro-meter and nano-meter scales.

Rennert *et al.*, 2014 conducted SEM-EDS study of a nodule from a Bg horizon of a Stagnosol prior to nano-SIMS study of that nodule. Their results revealed that heavier elements like Fe and Mn appeared lighter

and are centrally concentrated, forming linear structures that indicate layers of metal oxides, developed through soil's oxic/anoxic cycles (fig 17).

The formation of these oxides reduces pore space and cements quartz and silicates. And the distribution of Fe (red) and Mn (blue) (figure: 18) within region 1 [figure:17 (b)] showed element distribution mapping (EDS) of Fe and Mn accumulation though spatial resolution limits precise localization. Further the precise localization was shown by nano-SIMS images (figure:12).

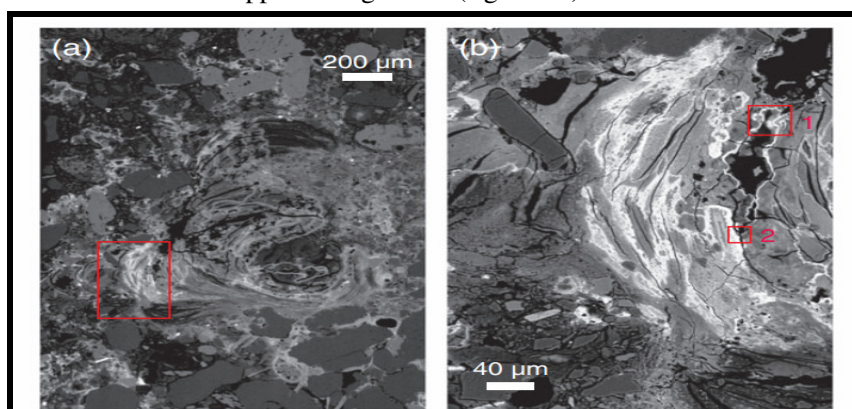


Fig. 17: Backscattered-electron images of a thin section from the nodule from a Bg horizon of a Stagnosol. (b) Shows the magnification of the section marked in (a). The sections marked in (b) were used for further EDS and Nano-SIMS measurements. (Rennert *et al.*, 2014)

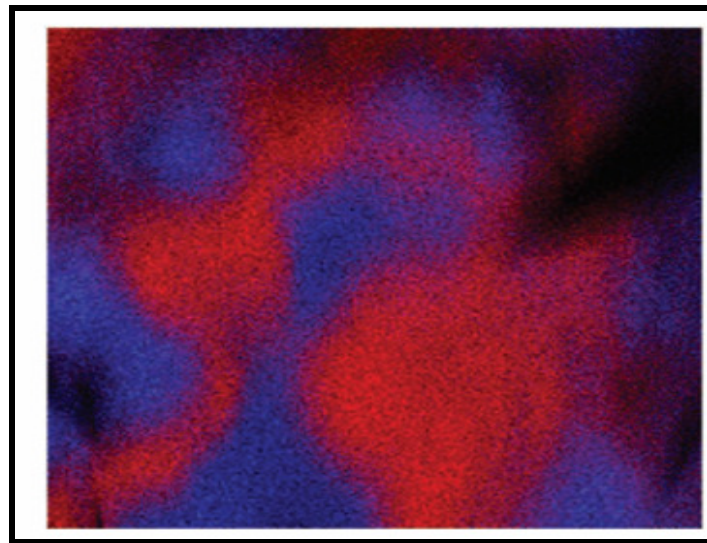


Fig. 18: Distribution of Fe (red) and Mn (blue) within region 1 (figure 17) on a thin section of a nodule from a Bg horizon of a Stagnosol measured by SEM-EDS. The dimension of the map (height \times width): $36.6 \times 35.5 \mu\text{m}$. (Rennert *et al.*, 2014)

A variety of synchrotron-based techniques (i.e., techniques that benefit from the very high flux densities of radiation generated by synchrotron radiation sources) have been applied along with nano-SIMS to the study of OMAs. The spatially resolved chemical information and the ability to interrogate microscopic samples have helped to understand the heterogeneity of OMAs.

Remusat *et al.* (2017) investigated samples from a decadal ^{15}N litter labelling experiment in a temperate

forest located at Ebrach in Germany & demonstrated the potential of Nano-SIMS to image intact soil particles and detected spots of isotopic enrichment even at low levels of ^{15}N application. They showed how microsites of isotopic enrichment detected by Nano-SIMS can be speciated by Scanning transmission X-ray microscopy combined with near-edge X-ray absorption fine structure spectroscopy (STXM-NEXAFS) performed on the same particle. (figure: 15 & figure 19)

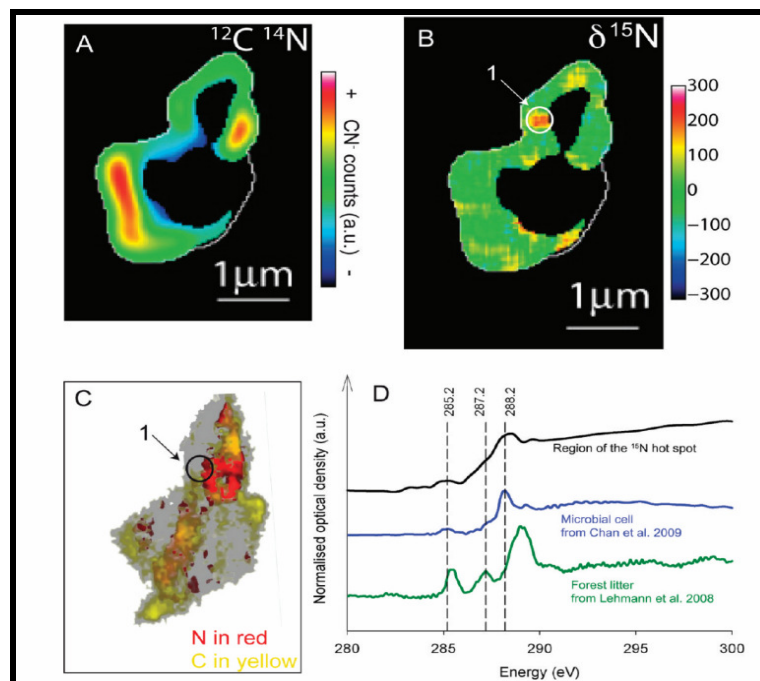


Fig. 19: Joined Nano-SIMS /STXM study of a soil aggregate with a ^{15}N hot spot collected from forest top layer in Ebrach, Germany where ^{15}N labeled leaves were deposited 12 years before sampling. (Remusat *et al.*, 2017)

Fig 19 (A): ^{26}C N image & Fig (B): $\delta^{15}\text{N}$ image of the same grain. The point (1) indicates a ^{15}N -rich hot spot, $\delta^{15}\text{N} = 213 \pm 132\%$. STXM C and N imaging is reported in (C). Carbon-NEXAFS (panel D) shows that the litter-derived- ^{15}N (region 1). C-NEXAFS Spectrum Showed a broad absorbance band from 287.8 eV to 288.9 eV, peaking at 288.5 eV. This indicated carboxyl-C with amide type features, suggesting a mixture of proteins, amino-sugars, and acid-containing polysaccharides.

The ^{15}N -rich hot spot is likely microbial material rather than fresh plant litter which was supported by comparison with pure microbial cells, indicating the ^{15}N enrichment originates from microbial metabolites decomposing the ^{15}N -rich litter. These observations suggested that microbial processing of litter- ^{15}N favoured its retention in soil through interactions with mineral surfaces and prevented it from being continuously cycled and eventually diluted, released, and lost as inorganic ^{15}N . (Remusat *et al.*, 2017).

Vogel *et al.* (2014) obtained similar results, utilizing the isotopic sensitivity of a NanoSIMS to confirm the heterogeneity of OMAs. Their findings supported the long-standing paradigm, based on bulk analysis, that only a small portion of mineral surfaces are actually coated with organic compounds.

It can be concluded that Nano-SIMS can effectively investigate isolated soil microstructures, identifies organic and mineral components in microaggregates and detects isotopic labels, even in weakly labeled samples. The presence of label-rich hot spots in long-term experiment indicated that labeled

organic matter can be preserved in soil for extended periods & combining STXM-NEXAFS with Nano-SIMS provides more robust conclusions than Nano-SIMS alone.

Synchrotron-based FTIR Spectro-microscopy also has the potential to reveal information about OMAs. This technique has the advantage of being able to detect both the mineral and organic phase at the same time. This is also the limitation of this approach as it means that understanding the specific structural feature associated with a particular resonance is difficult.

Yu & Liu (2022) identified the types and spatial distribution of functional groups of mineral-associated organic matter in contrasting fertilization treatments using high-resolution spatially-related SR-FTIR Spectro-microscopy (figure 20). This technique collected spectra through soil particles with a fine scan model of $1\mu\text{m}$ step $^{-1}$. They study found that the N-H stretching of amines ($\sim 3,200\text{--}3,400\text{ cm}^{-1}$), C-H stretching of lipids ($\sim 2,920\text{ cm}^{-1}$), and aromatic C=C vibrations and amide N-C stretch ($\sim 1,650$ and $1,530\text{ cm}^{-1}$, respectively) were stronger in long-term manure-fertilized soil (M) compared to long-term inorganic fertilized soil (NPK). Strong absorbance of functional groups from secondary minerals ($<950\text{ cm}^{-1}$) indicated that secondary minerals (e.g., Fe-O and Al-O) play a vital role in SOC storage. Nano-SIMS images (figure 11) supported these findings, showing that secondary minerals were co-localized with amines, lipids, and proteins in manure-fertilized soil but only with proteins in inorganic-fertilized soil.

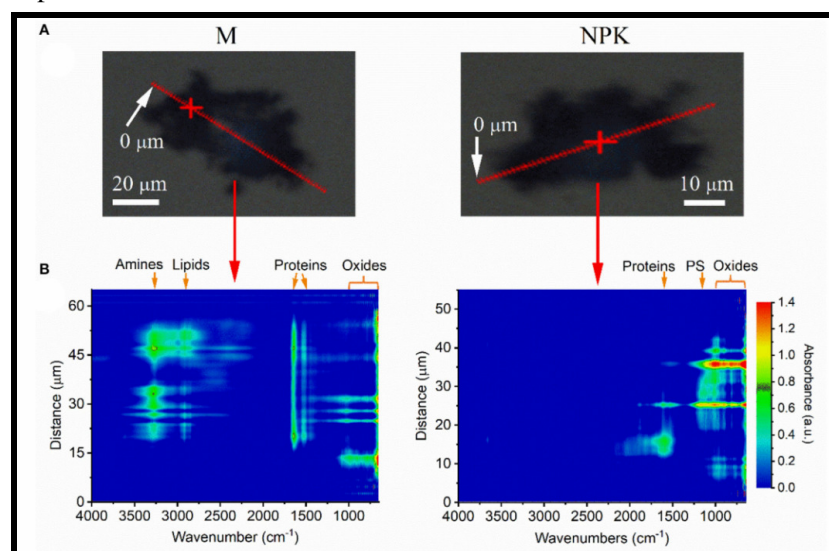


Fig. 20: Optical images (A) and the spatially related SR-FTIR- Spectro-microscopy (B) of soil water dispersible colloids collected from a long-term field experiment (1986- 2018) from the Institute of Red Soil, Jinxian Country where treatments were (1) without fertilization (Control), (2) farmyard manure fertilization (M), and (3) inorganic fertilization as nitrogen, phosphorus, and potassium fertilizer combination (NPK). (Yu & Liu, 2022)

Hence Yu & Liu (2022) from the results shown in figures 11 and 20, concluded that long term (32 years) application of manures could increase the bioavailability of minerals to C & accelerate the formation of organo-mineral association. Hence the fertilization practices could alter the potential preservation capabilities of Al & Fe minerals for C & Organic matter.

XPS (X-ray Photoelectron Spectroscopy) is a surface-sensitive quantitative spectroscopic technique that measures the elemental composition, empirical formula, chemical state, and electronic state of the elements within a material. It works by irradiating a material with X-rays and measuring the kinetic energy and number of electrons that escape from the top 1–10 nm of the material's surface. The Al 2p_{3/2} XPS measurement specifically focuses on the binding energy of the electrons in the 2p_{3/2} orbital of

aluminum atoms in the sample, providing information about the chemical state and environment of aluminum in the material.

Fe K-edge XANES analysis involves measuring how the X-ray absorption of iron changes as a function of energy near the K-edge. This technique provides insights into:

1. Oxidation State: The energy position and shape of the absorption edge can indicate the oxidation state of iron (e.g., Fe²⁺ vs. Fe³⁺).
2. Coordination Environment: The local atomic structure around the iron atoms, such as the types and number of neighboring atoms, and the symmetry of the surrounding environment.
3. Electronic Structure: Information about the unoccupied electronic states and the distribution of electronic density around the iron atoms.

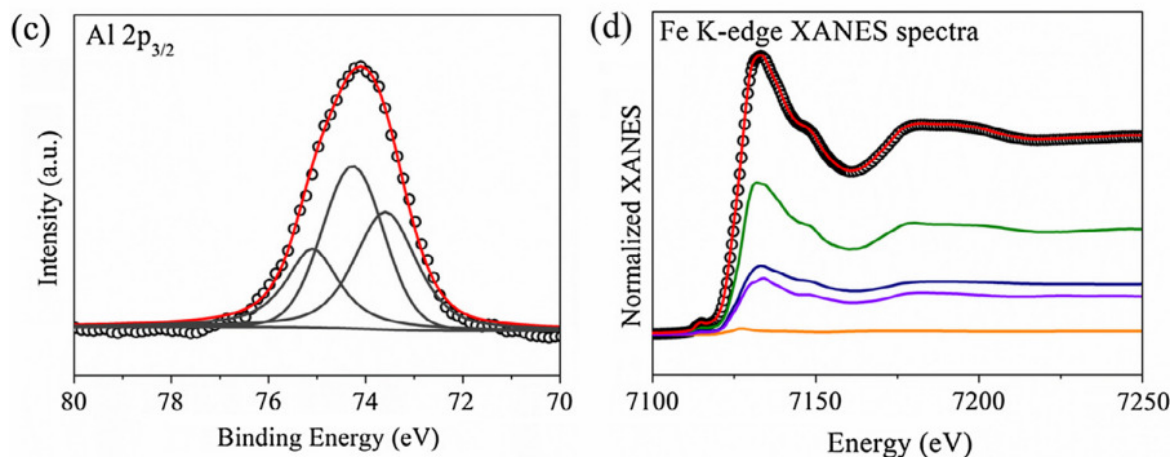


Fig. 20 : (c) XPS peak-fitting (Al 2p_{3/2}) images of the highly reactive Al minerals.
(d) The linear combination fitting (LCF) results of the Fe K-edge XANES spectra of the highly reactive Fe minerals. (Xiao *et al.*, 2015)

Xiago *et al.*, 2015 analyzed the functional groups and content percentages of highly reactive Al and Fe minerals in soil colloids using Al 2p_{3/2} XPS and Fe K-edge XANES. Al 2p_{3/2} XPS [see figure 14 (c)] revealed three main aluminum mineral groups: Al₂O₃/Al₂O₃-nH₂O (41.38%, e.g., allophane), AlO(OH) (35.63%, e.g., boehmite), and aluminum oxides (22.99%). Fe K-edge XANES (fig 14 (d)) through linear combination fitting with standard iron materials, identified the Fe mineral phases as crystalline goethite (53.27%), crystalline ferrihydrite (25.68%), crystalline hematite (19.44%), and inorganic ferrous oxides (1.61%).

Previous studies have showed that amorphous allophane and ferrihydrite had a stronger SOM

preservation capability than other crystalline mineral compositions, e.g., boehmite, goethite and hematite (Baker *et al.*, 2010; Lalonde *et al.*, 2012). This could be because with a shift in microbial metabolism and enzyme production, the meta-stable highly reactive Al and Fe minerals (e.g., allophane and ferrihydrite) could form stable organo-mineral bonds through anion and inner-sphere ligand-exchange reactions, thus being well-suited to physically protect the geometries which was supported by the nano-SIMS images (fig 13).

Confocal laser scanning microscopy (CLSM observation) shows the distribution of different biopolymers on soil OMAs where different fluorescent stains are used to label moist soil samples.

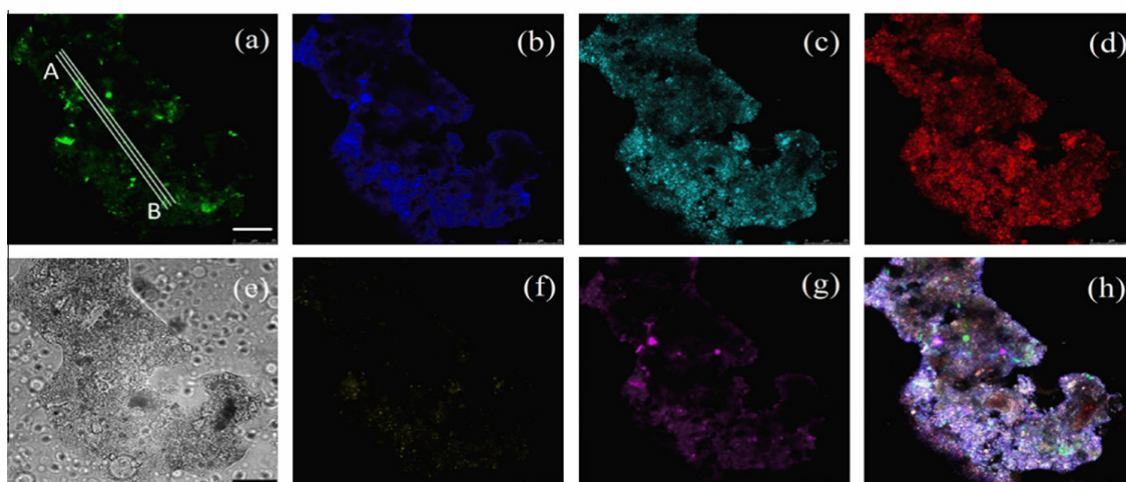


Fig. 21 : In situ spatial distribution of identifiable organic biopolymer images at the surface of the soil organo-mineral associations observed by CLSM at the micro-scale (155 X 155 μm) on a soil colloid obtained from a MNPK (swine manure plus chemical nitrogen, phosphorus and potassium) treatment site (1990–2013) conducted by the Chinese Academy of Agricultural Sciences, Qiyang, China. (Xiao *et al.*, 2015) (a) Proteins (green). (b) β -D-polysaccharides (cellulose) (blue). (c) α -polysaccharides (cyan blue). (d) Total cells (red). (e) Phase contrast photograph. (f) Lipids (yellow). (g) Dead cells (pink). (h) The combined image of individual images from (a) to (g).

The fluorescence staining results (figure 20) on soil colloids obtained from a MNPK (swine manure plus chemical nitrogen, phosphorus and potassium) treatment site (1990–2013) revealed significant chemical and spatial heterogeneity in the distribution of organic biopolymers at the soil organo-mineral association surface. Proteins were predominantly located at the core, while total and dead cells, β - and α -D-polysaccharides, and lipids were unevenly distributed in the outer layer. With this above CLSM observations (figure 20) along with nano-SIMS observation (figure 13) and XPS- XANES observation (figure 14), Xiao *et al.* (2015) concluded that mineral reactivity due to its various surface properties, heterogenous distribution and reactivity of biopolymers along with chemical heterogeneity of soil colloids and soil matrix combinedly influence the Complexity & heterogeneity in microenvironment of Soil OMAs. Hence there is a possibility that the regulation of highly reactive Al and Fe minerals through the appropriate management of organic fertilizer farming systems could help in efficient SOM preservation. (Xiao *et al.*, 2015; Yu & Liu, 2022)

Conclusion

Nano-SIMS is a technique that helps identify organic and mineral components in tiny soil aggregates and can detect small amounts of isotopic labels, even in samples that aren't strongly labeled. However, when Nano-SIMS is combined with other techniques like Scanning Transmission X-ray Microscopy (STXM) and Scanning Electron Microscopy (SEM) with Energy

Dispersive X-ray Spectroscopy (EDS), it provides more reliable results than using Nano-SIMS alone. Organic mineral associations (OMAs) play a crucial role in increasing soil organic matter (OM) storage, improving soil health, resilience, and supporting sustainable productivity. By using a combined imaging and spectroscopic approach, scientists can better understand what drives the persistence of soil organic matter (SOM) and the mechanisms that protect SOM. This helps in predicting changes in soil carbon storage in response to global environmental changes.

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