Supplementary information

Probing the nature of soil organic matter

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2.1 'Humification' refers to the synthesis of large molecules from decomposition products (Kononova 1966; Supplementary information). The study of 'humification' requires wet chemistry extraction methods such as alkaline hydrolysis (Kononova 1966) (Figure 1a). However, colorimetric or chromatographic analyses (Kononova 1966) following alkaline hydrolysis can only account for 30-70% of the OC (Rice 2011) with the original structure of SOM possibly altered during a range of secondary reactions (Kögel-Knabner 2000). Further, alkaline hydrolysis cannot distinguish among 'humic substances', 'non-humic substances', and products of secondary synthesis.

Alkaline hydrolysis usually involves the addition of a sodium hydroxide solution (less commonly potassium hydroxide) with a very high pH of about 13 to a soil sample (Kononova 1966). At this pH, most oxygen-containing functional groups in organic matter are deprotonated, making organic compounds bearing such groups much more soluble in water. There is a considerable proportion of organic matter that does not respond to the treatment, either for a lack of ionizable functional groups or because it was shielded from alkaline hydrolysis by mineral protection (Kleber and Lehmann 2019).

After adding protons to the solubilized organic materials, a dark solid precipitate commonly called 'humic acid' is quantified. The organic matter that remains soluble after reacidification is called 'fulvic acid'. The remaining irresponsive organic matter is commonly referred to as 'humin' (Baldock et al., 1992). Chemical and/or thermal degradation followed by colorimetric or chromatographic analyses of alkaline extracts (Kononova 1966) have been applied to detect aromatic functional groups, polysaccharides, proteins, or lipids in SOM and their molecular weights, optical and colloidal properties (Rich 2011). Pyrolysis gas chromatography mass spectrometry of these extracts can be confounded by the complexity of pyrolysis products derived from macromolecular structures, and detailed knowledge of the pyrolysis behaviour is required (Kögel-Knabner 2000).

Further, the available evidence does not support the formation of large-molecular-size and persistent 'humic substances' in soils (Lehmann and Kleber 2015). 'Humic substances' were also

considered to have greater aromaticity compared to bulk soil because of their higher content of polyaromatic molecules. In fact, pyrogenic carbon (Skjemstad et al., 1996; Lehmann et al., 2008; Rodionov et al., 2010) and microbial metabolites (not waste products, Chen et al., 1998; Staunton and Weissmann 2001; Liang et al., 2019) in soil can produce aromatic-rich alkaline extracts.

High resolution spectroscopic techniques such as synchrotron-based soft X-ray scanning transmission X-ray microscopy (STXM) showed that spectral properties of alkaline extracts are not found in soils at a nanometre scale (Schumacher et al., 2005; Lehmann et al., 2007, 2008; Chen et al., 2014). In fact, organic materials in alkaline extracts are assemblages of smaller compounds mimicking larger molecules (Myneni et al., 1999).

2.2 'Selective preservation' assumes that SOM is preferentially mineralized, leaving intrinsically 'stable' decomposition products behind (Melillo et al., 1982; Aber et al., 1990; Mueller and Kögel-Knabner 2009; Prater et al., 2020). In fact, provided with access and suitable conditions, soil microbial communities (and implicitly the associated enzymatic repertoire) can degrade a wide range of substrates in almost any soil including pyrogenic OC and polymers (Dungait et al., 2012; Nunan et al., 2015). SOM preservation mechanisms are, therefore, not linked to its chemical recalcitrance but rather to other biological, physicochemical, and structural factors (Schmidt et al., 2011), particularly its ability to associate with minerals (see later discussion).

2.3 'Progressive decomposition' reflects the concept of microbial processing of large plant biopolymers to smaller molecules (Trumbore 1997; Burdon 2001; Cotrufo et al., 2013). Progressive decomposition integrates the concepts of litter decomposition with protection and mineralization of SOM (Cotrufo et al., 2013). The chemical composition of 'humic substances' can be explained as the mixture of known plant and microbial compounds (Kelleher and Simpson 2006).

4.1.1 Solid state 13C nuclear magnetic resonance (NMR)

Pre-treatment of soil using hydrofluoric acid is often used to minimize the loss of signal in the presence of these minerals which can compromise the detection accuracy in iron-rich soil such as Ferralsols/Oxisols (Kögel-Knabner 1997; Hedges et al., 2000; Begaudeau et al., 2012). Nevertheless, recent efforts used this signal loss to probe the association between paramagnetic Fe^{3+} and distinct C forms (carboxylic, aromatic, O/N-alkyl, and alkyl C components) directly in bulk soils without any pre-treatment using CPMAS ^{13}C NMR (Possinger et al., 2020b). The OC-Fe^{3+} interactions can be interpreted as the proton relaxation time decreases with increasing Fe content in the NMR spectrum (Schöning et al., 2005).

5 Combined in situ approaches to study the space-time-composition continuum

Comparisons between similar techniques need to be drawn to align specific methods with the appropriate question. For bulk analyses, firstly, in NEXAFS, it is generally the characteristic fluorescent X-rays that are emitted and analyzed, but in XPS it is the kinetic energy of the ejected electrons (photoelectric effect) that are measured. Secondly, for XPS, the energy of the incident X-ray beam is constant (fixed), which contrasts with NEXAFS where the energy is progressively increased in small increments across the absorption edge (for NEXAFS, this results in highly detailed spectra for the individual element of interest). As a result, the comparative advantage of XPS is that it is possible to examine multiple elements simultaneously. However, this also means that XPS is considerably less sensitive to the bonding environment and hence provides considerably less information regarding speciation than NEXAFS. For two-dimensional analyses, EELS has greater spatial resolution but lower spectral resolution compared with NEXAFS-STXM and NMR (Figures 2

and 3). Further, one caveat of imaging is the average of z dimensions of the samples. In the case of sectioning for IRM, STXM, TEM and EELS or ion sputtering for XPS and NanoSIMS, only the average over the thickness of the section or the upper surface (*e.g.* 100 nm) was calculated which prevents a better understanding of organo-mineral and organo-organic interfaces which can be in the range of single-digit nanometers. Future research should consider combining tomography to improve the understanding of interfacial chemical structure of SOM.

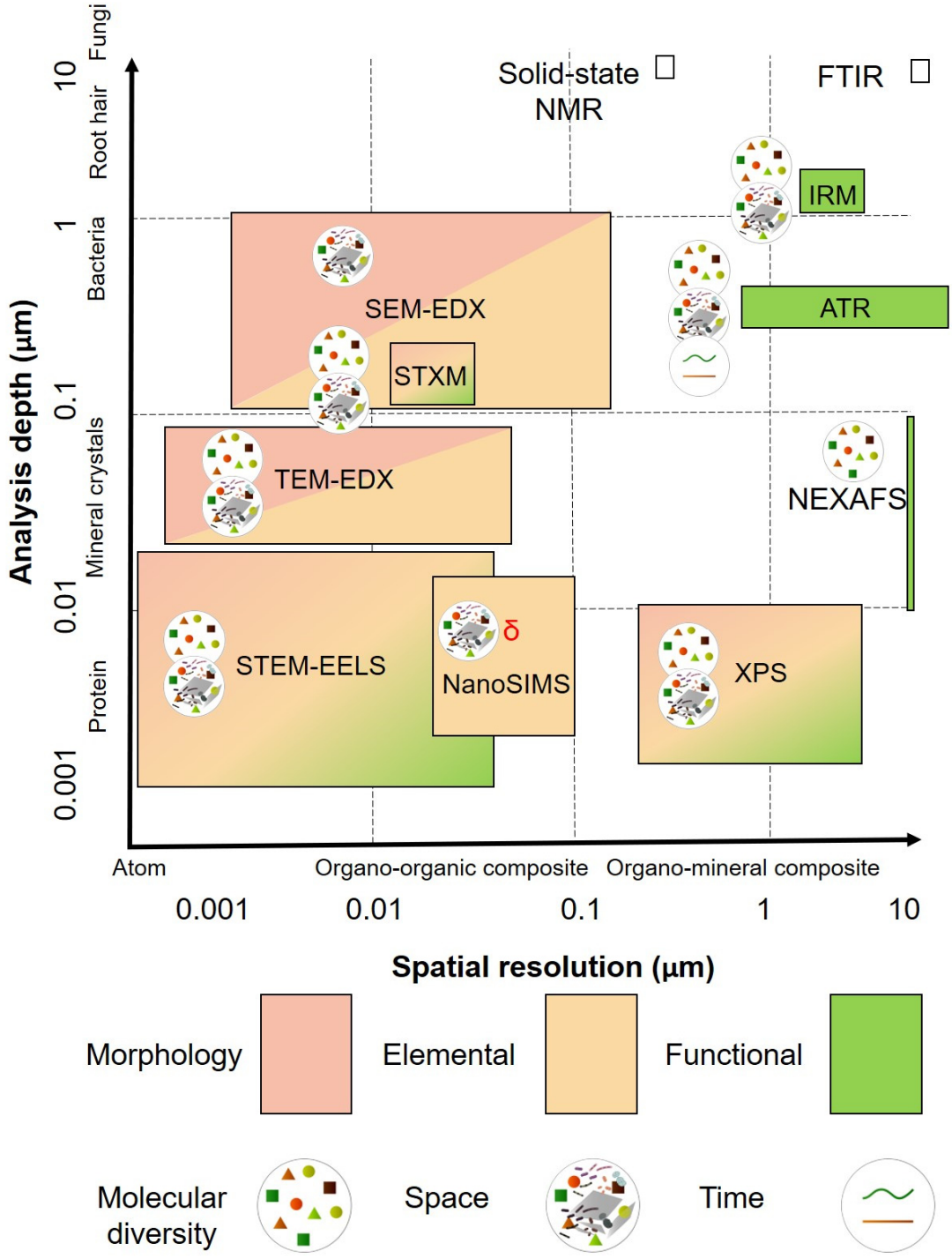

Figure S1. High-resolution *in situ* investigations of micro- and nanoscale spatial arrangement of molecular diversity (organic C functionalities) over time. Morphological, elemental and functional features of organo-mineral and organo-organic interactions can be probed via a combination of interdisciplinary techniques. Temporal assessment is currently not available at a nanometre and submicron scale using X-ray- or electron- based techniques which are often performed on dehydrated samples under cryogenic and ultra-vacuum conditions (1×10^{-10} mbar). Recent development at the Canadian Light Source enables cryo-STXM under ambient air, He, and low vacuum (~10^{-6} mbar; Hitchcock 2015). Morphological and elemental information are less available at microscale (i.e. > 1 µm laterally and vertically).

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