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(Article begins on next page)

**Iron speciation in organic matter fractions isolated from soils amended
with biochar and organic fertilizers**

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Abstract

The role and distribution of iron (Fe) species in physical soil fractions has received remarkably little attention in field-scale systems. Here, we identify and quantify the Fe phases in two fractions (fine sand, FSa, and fine silt and clay, FSi+Cl) isolated from an agricultural soil non-amended and amended with different organic materials, by Fe K-edge extended X-ray absorption fine structure (EXAFS) spectroscopy. Linear combination fitting and wavelet transform of EXAFS data revealed noticeable differences between unamended FSa and FSi+Cl fractions. Specifically, the FSi+Cl fraction was mainly characterized by ferrihydrite (48%) and Fe(III)-soil organic matter (SOM) complexes (37%), whereas in the FSa fraction ferrihydrite still represented a major phase (44%), with a lower contribution from Fe(III)-SOM (18%). In the FSa fraction, the addition of the organic amendments resulted in an increase of Fe-SOM complexes (31-35%) and a decrease of ferrihydrite (28-29%). By contrast, in the amended FSi+Cl fractions, the added organic matter led to negligible changes in percent ferrihydrite. Therefore, regardless of the amendment type, the addition of organic matter to soil increased the capability of the coarse fraction (FSa) to stabilize organic carbon, thus pointing out that the role of FSa in C sequestration in agricultural soils at a global scale may be overlooked.

ha eliminato: are of fundamental importance in regulating organic carbon (C) biogeochemistry. However, despite the relevance of the topic and extensive investigation at the laboratory scale, it

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INTRODUCTION

Understanding soil organic carbon (C) cycling and its response to climate change requires identification of the mechanisms involved in the buildup of soil organic matter (SOM) pools with long residence time.¹⁻⁶ Soil scientists are recently asked to provide concrete answers to two major questions with regard to global issues concerning greenhouse gases emissions: (1) can agricultural soils sustainably increase their total sequestered organic C contents,³ and (2) how will soils respond to the expected global warming trend.⁴ These issues are a challenge because “Sustainable agriculture is not a luxury”.⁵

Soils may contain a broad range of iron (Fe) mineral species, including clay minerals, Fe-SOM complexes, and polymeric Fe (oxyhydr)oxides, and their reactive surfaces drive C stabilization.^{6,7} In particular, it has been observed that SOM and low crystallinity short-range-order (SRO) Fe mineral contents are positively correlated.^{8,9} Land use changes and agricultural practices, including the application of organic amendments, can modify the physico-chemical conditions related to mineral stability, affecting the reactive surfaces of organo-mineral complexes and leading to the dissolution of already existing minerals and the formation of others.^{10,11} This leaves still several open questions about the influence of Fe species and abundance on SOM stability,^{12,13} especially in agricultural soils which are commonly subject to amendment with different organic fertilizers (including biochar).^{14,15} Quantifying those species in different particle size fractions of soils through advanced spectroscopic techniques can constitute an additional rapid and nondestructive approach to sequential extraction methods traditionally used to quantify the stability of organic C associated to Fe phases.¹⁶

One of the most valuable tools to study stabilization mechanisms of SOM is its fractionation by particle size, as size fractions (sand, silt and clay-sized) contain both mineral particles and SOM and represent the organo-mineral units. The SOM in the fine-

silt and clay (FSi+Cl) fraction is stabilized by electrostatic interactions with clays, hydrogen bonds, precipitation by polyvalent cations (metal bridges) and association with aluminum (Al) and Fe oxides.¹⁷ On the opposite, the fine sand fraction (FSa) is supposed to play a secondary role for organic C partitioning in soils, due to its low surface area and reactivity.¹⁸ The adsorption phenomena and cation exchange capacity of these coarse fractions can be affected by coatings with Fe oxides through electrostatic forces, Van der Waals interactions, and Fe-O-Si bonds.¹⁹

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Moreover, in order to advance the understanding of SOM stabilization mechanisms, we need to go beyond the size fractionation and combine it with an exhaustive molecular characterization not only of the SOM, but also of the mineral phases (including Fe minerals). In fact, limited research has been performed to clarify the complex interdependence between different Fe mineral phases and SOM at an atomic scale using advanced spectroscopic techniques.²⁰ Moreover, although biochar, alone or in association with other organic amendments (*e.g.*, compost), has been described as a C sequestration mechanism,^{15,21,22} little is known about its role on the associations between SOM and Fe-bearing minerals in naturally occurring soil fractions.

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Advanced spectroscopic techniques provide both physical and chemical information at the molecular level to help understand soil organic C (SOC) and Fe interactions. For example, Extended X-ray Absorption Fine Structure (EXAFS) spectroscopy has been used to characterize Fe(III) in organic soils under different pH ranges and with varying natural Fe content,^{20,23} as well as to investigate Fe speciation in soils and soil aggregates.²⁴

Recently Fe EXAFS has identified the role of fertilization practices on Fe speciation, but this research focused mainly on soil colloids.^{11,25,26} Xiao et al.¹¹ studied the reactive mineral species and their associations with C affected by inorganic and organic (*i.e.*, manure application) fertilization and suggested a greater C loading by Fe minerals under

organic rather than chemical fertilization. Fe speciation in bulk soils, soil horizons and aggregates, and the Fe spatial distribution in the aggregates, is controlled mainly by weathering, pH, redox potential, and organic ligands activity.^{24,27} Moreover, aggregate and mineral particle size can affect both SOM storage mechanisms and Fe speciation, and thus, the role of Fe-bearing minerals in organic C retention. Pairing traditional particle size fractionation with advanced spectroscopic allows for determination of Fe species in each size fraction. This is important because the role of SOM in Fe hydrolysis and in the formation of various hydrolyzed Fe species in natural soil systems is still not well-known.

The application of Fe EXAFS in structurally and elementally complex bulk soils and soil fractions is still a challenging data analysis problem. In fact, during EXAFS analysis of trace and transition metals in soil and geochemical systems, a detailed analysis of the second shell is extremely difficult when several different types of elements are present. This is due to the overlap of the scattering photoelectron waves during the absorption process,²⁸ and particularly true when the elements are comparatively high and low in atomic weight, such as C and Fe. For this reason, we employed a six-fold approach to both quantitatively and qualitatively evaluate the standards and spectral fitting in order to produce conservative results and minimize potential overinterpretation of the data.

Consequently, coupling EXAFS analyses (e.g., linear combination fitting (LCF) and Wavelet transformation (WT)) with particle size fractionation would allow examining how Fe speciation varies in soil samples of different particle sizes. In particular, LCF is a process in which the spectrum of the samples is determined using the other spectra of well characterized standards, whereas WT provides useful information resolving simultaneously data in both wave space (k-) and interatomic distance (R-) space, and the resulting k-R maps are helpful in improving the fitting model and understanding elemental speciation in the sample.²⁹⁻³¹

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In this work, we used Fe EXAFS (*i.e.*, the classical LCF analysis paired with WT) to investigate the abundance and composition of Fe minerals in two different size fractions having opposite features, *i.e.*, fine sand (FSa; 200-50 μm) and fine silt and clay (FSi+Cl; <20 μm), isolated from agricultural soils as affected by different organic amendments: biochar (BC), municipal solid waste compost (MC) and the simultaneous application of both (BC+MC).

MATERIALS AND METHODS

Soil samples collection, analysis and physical fractionation

The site selected for this research was located in the experimental farm “La Poveda” (Spanish National Research Council, CSIC), Arganda del Rey, Madrid, Spain (40190 N, 3290 W, 534 m above sea level). The site was characterized by a Mediterranean climate, with an average annual rainfall of 436 mm and average annual temperature of 14 °C. The soil had a clay loam texture and was classified as a Xerofluvent.³² The properties of the top soil (0–15 cm) and amendments are fully reported elsewhere,^{15,33} with main soil features summarized in Table S1. The experiment was set up as a randomized complete block design with two factors, namely BC and organic fertilizer, with four replicates. In particular, a control treatment (no amendment) and a BC application at a rate of 20 t ha⁻¹ were combined with no organic fertilization, and a MC at a rate equivalent to 75 kg potentially available N ha⁻¹. Biochar and MC were broadcast in late October 2012 and immediately incorporated into the upper 15 cm of soil with a rotary tiller. Soil samples representative of the plow layer (0–15-cm depth) were collected randomly from each plot (5x3 m²) in late June 2013, gently crushed and passed through a 2-mm sieve for physical fractionation. Total Fe concentration in soil and amendment samples was determined by

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Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) after digestion with nitric and perchloric acid.^{33,34}

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A physical size fractionation by ultrasonic dispersion and wet sieving was performed, allowing for splitting particles into four different size fractions: coarse sand (CSa: 2000-200 μm diameter), fine sand (FSa: 200-50 μm), coarse silt (CSi: 50-20 μm) and fine silt and clay (FSi+Cl: <20 μm).³⁵ Briefly, 15 g of each sample were put into 50 mL vials and

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filled with deionized water to 3/4 of their volume. Samples were then subjected to vertical agitation (20 rpm) for 60 minutes and to ultrasonic dispersion of the soil particles at an energy input of 1020 J mL⁻¹ using a Branson 45 sonifier (100 W output, 10 minutes, 60% of the total amplitude). The derived suspensions were then wet-sieved through a set of three sieves (200 μm , 50 μm and 20 μm mesh) splitting particles into four different size fractions. The fractions retained by sieves (CSa, FSa, CSi) were recovered, quantitatively transferred to pre-weighted vials and dried at 60 °C to constant weight. The particles passing through the last sieve (<20 μm : FSi+Cl) were brought to 1 L with water. Two mL of saturated solution of potassium alum were added, and the suspension was left to stand refrigerated for 2 days, until complete sedimentation of the solid particles. The overlying water was siphoned off and discarded. The sediment was recovered by centrifugation (15 min at 2500 g), transferred to a pre-weighed flask and dried at 60 °C to constant weight. In the present research, we used and compared only the FSa and FSi+Cl fractions, representing them two fractions with opposite features in terms of specific surface area (SSA), reactivity and, in turn, potential influence on the SOC turnover time.

Organic C and total N

Total organic C and N contents were determined by dry combustion using a Thermo Flash 2000 NC Soil Analyzer. Each sample was ground with a ball mill and subjected to acid

fumigation before the analysis to remove carbonates.³⁶

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Iron K-edge Extended X-ray Adsorption Fine Structure (EXAFS) spectroscopy

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Freeze-dried and powdered sample material (*ca.* 15 mg) was pressed into pellets and sealed with Kapton® tape and mounted in a sample chamber at room temperature. X-ray absorption spectroscopic analyses of FSa and FSi+Cl fractions were conducted at the

XAFS beamline at Elettra Sincrotrone (Trieste, Italy).^{37,38} Fe EXAFS spectra were

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collected in transmission mode using Si (111) monochromator, which was calibrated to the first-derivative maximum of the K-edge absorption spectrum of a metallic Fe foil (7112 eV). The foil was continuously monitored to account for small energy shifts (<1 eV) during the sample measurements. Higher harmonics in the beam were eliminated by

detuning the monochromator by 40% of its maximal intensity. LCF analyses of k^3 -

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weighted Fe and EXAFS spectra were performed over a k -range of 2-10 $\text{\AA}^{-1}$ with the E_0

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of all spectra and reference compounds set to 7132 eV. EXAFS data processing (*e.g.*, background removal, normalization, glitch removal at 7250 and 7600 eV) and spectra generation were carried out with the software Athena.³⁹ Fourier transform and k -weighted

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chi spectra were used to create wavelet transforms using the Igor Pro wavelet transform script developed by Funke et al.³⁰

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A suite of fitting techniques designed to identify the major Fe components in the studied samples was employed to analyze the synchrotron-based data. In fact, due to both the sample complexity and the experimental limitation on the number of samples that can be analyzed by EXAFS analysis, a six-fold approach was employed to select specific EXAFS standards for fitting, including (1) Principal Component Analysis (PCA), (2) Target Transformation (TT) which produces (3) SPOIL values for each standard, a (4)

scree plot, (5) visual inspection of principal components and finally (6) an F-test to determine the most appropriate number of standards.

X-ray diffraction (XRD) analysis

X-ray powder diffraction measurements were carried out using a synchrotron source at the MCX (Materials Characterisation by X-ray diffraction) beamline at Elettra Synchrotron (Trieste, Italy)^{40,41} using a wavelength, $\lambda = 0.9537 \text{ \AA}$ ($E = 13 \text{ keV}$). The beamline is equipped with a diffractometer which has a four-circle goniometer. The samples were loaded in borosilicate capillaries with a diameter of 0.5 mm, which were spun during the measurement at a speed of 180 rpm to ensure a randomized orientation of the crystallites in the sample. XRD patterns were collected in the $1.5\text{--}60^\circ 2\theta$ range. Diffracted intensities were recorded using a scintillator detector behind a set of receiving slits. The exposure time for each point was 1 s. The patterns have been analyzed using the computer program QualX2.0.⁴² Semi-quantitative analysis was conducted using the normalized reference intensity ratio (RIR) method⁴³ and RIR values in the powder diffraction database of the International Centre for Diffraction Data. All calculations were performed using X'Pert HighScore 2.2e (Malvern Panalytical, Malvern, UK).

RESULTS AND DISCUSSION

Mineralogy and concentration of organic C and total N of the fractions

The FSa and FSi+Cl fractions show a similar composition in terms of main mineral phases, with some differences in terms of relative percentages. In particular, quartz is much more abundant in the FSa (67-76%) compared to the FSi+Cl (34-58%), whereas muscovite is higher in FSi+Cl (8-15%) versus FSa (2-4%) (Table 1 and Figure S1).

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Smaller differences between the two fractions can be observed also for calcite and chlorite.

Organic C and total N contents of the examined fractions is shown in Table 2. Organic C content ranges from 3.5 to 9.1 g kg⁻¹ in FSa, and from 18.1 to 28.2 g kg⁻¹ in FSi+Cl. Total N varies between 0.26 and 0.41 g kg⁻¹ in FSa, and between 1.9 and 2.3 g kg⁻¹ in FSi+Cl. In all the cases, the lowest values are ascribed to the unamended soil (UN), whereas the soil amended with BC+MC contains the highest organic C and total N contents, in both FSa and FSi+Cl fractions. Therefore, in both FSa and FSi+Cl fractions, organic C and total N contents followed the order BC+MC > BC > MC > UN. In agreement with a previous work,¹⁵ BC combined with compost promote C stabilization in organo-mineral complexes by chemical binding between organic inputs and mineral particles.

Synchrotron-based data analysis

Methods for synchrotron-based data acquisition and analysis, PCA (Table S2 and Figure S2), TT, SPOIL values (Table S3), LCF (Table S4) of EXAFS data, as well as standards description, are reported in the Supplementary Material.

Linear combination fitting

LCF results (Figure 1 and Table S4) revealed the dominant species identified as major components by the fits. The Fe EXAFS data fitting showed that Fe phases in unamended FSa and FSi+Cl fractions are noticeably different. The fine fraction (FSi+Cl) of the unamended soil was mainly characterized by ferrihydrite and Fe(III)-SOM (48 and 37% of the fitting components corresponding to experimental spectra of model compounds, respectively). Low molecular weight organic acids constitute an important part of labile

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ha eliminato: A suite of fitting techniques designed to identify the major Fe components in the studied samples was employed to analyze the synchrotron-based data. In fact, due to the experimental limitation on the number of samples that can be analyzed by EXAFS analysis, a six-fold approach was employed to select specific EXAFS standards for fitting, including (1) Principal Component Analysis (PCA), (2) Target Transformation (TT) which produces (3) SPOIL values for each standard, a (4) scree plot, (5) visual inspection of principal components and finally (6) an F-test to determine the most appropriate number of standards.

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C and can be subjected to minerals and metal cation binding mechanisms;⁴⁴ among them, citrate is one of naturally occurring organic ligands existing as microbial metabolite and in root exudates.⁴⁴ Fe(III)-citrate is generally used as an analog model compound for Fe-SOM complexes because it consists of Fe bound directly to organic C.⁴⁵ In the coarse fraction (FSa), ferrihydrite represented the main phase (44%), followed by chlorite (26%) and with less significant contribution from Fe(III)-SOM (18%), here identified in the LCF with the Fe(III)-citrate standard. Our results agree with Asano and Wagai⁴⁶ who suggested that larger size fractions are increasingly dominated by primary mineral crystals and smaller size fractions are more dominated by the micron- and submicron-sized composites enriched in low crystallinity SRO minerals and organo-metal associations.

In the amended soils, ferrihydrite was prominent in all FSi+Cl fractions (47, 45, and 46% in BC, MC, and BC+MC amended soils, respectively), showing levels similar to those found in the same fraction isolated from the unamended soil (48%) (Table S4 and Figure 1). The remaining Fe phases were composed of clay minerals and Fe(III)-silicate, represented mainly by chlorite (21, 18, and 19% in BC, MC, and BC+MC amended soils, respectively). The percentage of Fe(III)-SOM was higher for the amended soils (44, 43 and 41% in BC, MC, and BC+MC amended soils, respectively) than for the unamended soil (37%) (Table S4). The FSa fraction of amended soils was characterized by higher chlorite contents (25-36%) and lower contents in ferrihydrite (28-29%) compared to that of the unamended soils (26 and 44% of chlorite and ferrihydrite, respectively). Finally, FSa fractions in amended soils showed higher Fe(III)-SOM contents (>30%) compared to those in the unamended soil (18%). It is to note that the fits of the FSi+Cl fractions consistently totaled >100%, whereas the FSa fractions consistently <100%.

The Fe concentration in BC and MC amendments was 0.97 and 8.7 g kg⁻¹, respectively, resulting in an Fe input to soil ranging between 11 and 103 mg Fe kg⁻¹ soil.

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327 Therefore, the increase in the proportion of Fe-SOM complexes with the organic
 328 amendments in both FSa and FSi+Cl fractions is promoted by the addition of organic
 329 materials and not due to a further, significant addition of Fe. In fact, in synthetic systems,
 330 it has been shown that SOM complexes with Fe(III) can hinder the transformation of
 331 Fe(III) into ferrihydrite.^{47,48} Specifically, the sorption of SOM and some organic acids
 332 (i.e., citric acid) on the ferrihydrite surface may inhibit the crystallization of further Fe
 333 oxide phases.⁴⁹ Additionally, the organic acids may complex Fe and promote Fe oxide
 334 dissolution and complexation. Xiao et al.,¹¹ investigating the same, single soil type but
 335 under different fertilization treatments, found that reactive Fe minerals were mainly
 336 composed of less crystalline ferrihydrite in the organic manure-treated soil and more
 337 crystalline goethite in the chemically fertilized soil, while Xu et al.⁵⁰ reported that the
 338 presence of low molecular weight organic acids was responsible of the further increase
 339 of SRO minerals content. To study the role of organic inputs on the formation of reactive
 340 minerals, Huang et al.²⁵ simulated the formation of SRO Fe minerals adding oxalic acid
 341 to soil colloids isolated from a long-term experiment in which swine manure was used as
 342 fertilizer. They confirmed that low molecular weight organic acids may incorporate into
 343 the structure of SRO minerals, thus inhibiting the further growth of these minerals. The
 344 effectiveness in suppressing crystallization can depend on whether and how strongly the
 345 organic acid adsorbs onto ferrihydrite and how strongly it complexes with Fe(III) in
 346 solution. Additionally, the extent by which the adsorption of organic molecules prevents
 347 the dissolution of ferrihydrite depends on the degree of surface coverage.⁵¹ In a previous
 348 sorption and desorption experiment,⁵² we proved that SOM in the FSa fraction can be
 349 more strongly stabilized by Fe addition than in the FSi+Cl. The reaction with Fe(III) may
 350 have caused two opposite effects on SSA; either a decrease in SSA mainly occurring in
 351 the FSi+Cl fractions and probably due to flocculation, or an increase of SSA values,

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probably due to newly formed Fe surface coatings.⁵³ The increased reactivity of FSa fractions might constitute an explanation for the higher SOM complexation to ferrihydrite in the FSa fraction.

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Organic matter-mineral complexation reactions in soils

Organic ligands are known, through surface adsorption, to either accelerate or retard dissolution, and this depends on the weakening of the Fe-O bond by organic ligands.⁵¹

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Mikutta et al.⁵³ found that the coprecipitation of polysaccharides with Fe(III) (oxyhydr)oxides can increase the ligand-accessible surface area of ferrihydrite, enhancing

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the dissolution of ferrihydrite by low molecular weight ligands. Therefore, the decrease in the ferrihydrite component observed in amended FSa fractions using LCF (from 44%

in the unamended to 28% in the BC+MC amended soils) is in agreement with Mikutta et al.⁵³ This may also suggest that the addition of organic amendments enhances Fe oxide

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dissolution. In the FSi+Cl fraction, the interaction between freshly added amendments can lead to surface site blockage and to the formation of more aggregated particles and, consequently, to a lower ferrihydrite dissolution. This is a competing hypothesis to the increase in Fe oxide dissolution and likely both processes may occur in the samples.

Mikutta et al.⁵⁴ reported that in systems with abundant SOM, organic chelates (such as

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hydroxybenzoic acids) can control the kinetics of ferrihydrite formation via Fe(III) complexation. The organic complexation effectively lowers the solution saturation state of inorganic Fe(III) species, which leaves less Fe(III) available for ferrihydrite

precipitation. Mikutta et al.⁵⁴ also illustrated that citrate decreased ferrihydrite particle

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size by impairing the polymerization of the octahedrons. Polymerization reaction occurs between partially hydrolyzed metal cations via –OH– or –O– bonding to form octahedral bridges by edge and corner linkages. Organic ligands surface adsorption may disrupt

those bridges or accelerate dissolution, weakening the Fe-O bond. Thus, Fe released into solution can give rise to Fe-SOM complexes.

The hypothesis of increased ferrihydrite dissolution after amendments in FSa rather than in FSi+Cl needs to be confirmed over time. By following ferrihydrite transformation dynamics, proving these mechanisms overtime seem to support the hindering role that organic amendments can play.

Analysis of peak positions

Fe EXAFS can be also used for qualitative analysis of the composition of Fe minerals. LCF results are drawn in Figure 2 and the fit of each sample, in dotted lines, is labeled with the R factor. Fe K-edge EXAFS spectra show similar features at $\sim 4.0 \text{ \AA}^{-1}$ (maximum 1), $\sim 6.5 \text{ \AA}^{-1}$ (maximum 2), $\sim 7.5 \text{ \AA}^{-1}$ (maximum 3), $\sim 8.5 \text{ \AA}^{-1}$ (maximum 4). The maximum at $7.5 \text{ \AA}^{-1}$ is characteristic of ferrihydrite (Figure S3) and becomes less intense in all amended FSa samples compared to the amended FSi+Cl samples. This qualitative difference can be illustrated quantitatively via the % ferrihydrite component, which is higher in FSi+Cl compared to FSa (47 vs. 29% in BC, 45 vs. 28% in MC, and 46 vs. 28% in BC+MC) (Table S4). The role of SOM in decreasing the amount of ferrihydrite component is supported by analyzing the FSa spectra (Figure 2). Compared to the control, the organic amendments are responsible of the shift of the maximum at $8.5 \text{ \AA}^{-1}$ towards the characteristic maximum at $8 \text{ \AA}^{-1}$ typical of Fe(III) citrate,⁴⁴ indicating the role of SOM in promoting Fe-SOM complexation. The already described quantitative decrease in the ferrihydrite component in the LCF results in the amended fractions is in agreement with the qualitative LCF data. Moreover, these results agree with Yang et al.⁴⁴ who observed the dissolution of ferrihydrite with the increased citric acid addition, and reported that the transformation of aqueous Fe^{3+} to ferrihydrite was mainly retarded with Fe/citric acid

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molar ratios <0.5. This peak shift in the amended soils is evident in FSa samples and not in FSi+Cl, in which the difference in Fe(III)-SOM ranged from 37% in the unamended soil to 44% in BC amended soil. The maximum at $4.0 \text{ \AA}^{-1}$ (*i.e.*, the peak 1, characteristic of ferrihydrite) remained unchanged, and its lower intensity in FSa indicates the lower percentage in ferrihydrite.

Wavelet transformation

Here, WT analysis was used to qualitatively test the presence of Fe back-scatterers in the second coordination shell of Fe. The Morlet wavelet transforms of k^3 -weighted Fe K-edge EXAFS spectra of the samples over a $R + \Delta R$ -range of 2.2-4.0 $\text{\AA}$ was calculated and the resulting wavelet plots compared with those of Fe(III) reference compounds (ferrihydrite, Fe(III)-citrate, chlorite).⁴⁵ Figure 3 contains the WT plots while Figure S4 the Fourier transform spectra of the standards. In the ferrihydrite plot, the Fe shell contributes a strong feature at 7-8 $\text{\AA}^{-1}$ and 2.75 $\text{\AA}$;⁵⁵ this feature is also indicated in Figure S6, where a broader range of eta (η) and sigma (σ) parameters, *i.e.*, two parameters allowing a more detailed analysis of how the Fourier transform peaks are distributed on the energy axis, were used. In the Fe(III)-citrate standard, the absence of Fe backscattering is accompanied by new features at distances of 2.0–2.5 and 3.1–3.7 $\text{\AA}$ and indicates back-scattering from lighter atoms, in agreement with single and multiple backscattering from C/O in the second and third coordination shells of tris(oxalato)iron(III).^{20,55-57} Moreover, these features appear at lower energies (3–4 $\text{\AA}^{-1}$) than those of Fe. The third standard shown in Figure 3 is chlorite. In general phyllosilicate minerals, which contain tetrahedral and octahedral sheets, can be described by three atomic backscattering paths: the first-neighbor O, second-neighbor octahedral Fe, Al, or Mg; and finally tetrahedral Si.⁴⁵ When multiple Fe oxyhydroxides and phyllosilicate

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minerals are present in the same sample, the Fe-Fe and other scattering elements at similar interatomic distances overlap. This makes it challenging to use shell fitting of EXAFS to identify specific elements in heterogeneous systems such as soils. However, other techniques, such as WT, can assist to distinguish different types of scattering elements.

The WT of the EXAFS data ($\eta=8$, $\sigma=1$) showed that both the intensity caused by light back-scatterers at low k-values and by heavier atoms at higher k varied among the samples (Figures 4 and S5). A general trend can be distinguished between the clay and sand samples, where the clay samples generally have more intense maxima near $8 \text{ \AA}^{-1}$. This is particularly true for the FSa from amended soils, where the maxima at *ca.* $7 \text{ \AA}^{-1}$ decreases in intensity. By looking at the Figure 4 from left to right, the decrease in the maxima in FSa samples is clear, where the peak changes from red in the unamended soil to yellow/green in the amended soils. The WT results support the LCF results by indicating that EXAFS spectra of the soil samples can be described as a mixture of Fe-bearing clay minerals, Fe (oxyhydr)oxides, and organically complexed Fe.

The WT plots of the unamended FSi+Cl (Figure 4a) and FSa (Figure 4e) samples indicate a Fe-Fe second shell contribution from ferrihydrite and chlorite. A contribution from Fe(III)-citrate at above $2.2 \text{ \AA}$ and $2\text{--}4 \text{ \AA}^{-1}$ is also evident. In the organic amended samples, the Fe-Fe signal remains the main feature in the FSi+Cl fraction (Figure 4b-d). A slight increase in the Fe-SOM signal contribution at above $2.2 \text{ \AA}$ and $2\text{--}4 \text{ \AA}^{-1}$ is also present, as reported in the LCF results. Fe-Fe backscatters in the second shell of FSi+Cl samples are in agreement with the results reported in LCF.

In all amended FSa fractions, the signal from the Fe-Fe second shell disappears (Figure 4f-h), mirroring the decrease in the ferrihydrite percentage from 44 (in the unamended soil) to 28-29%, whereas the increase of the feature from C backscattering is evident, and relates to the increase of Fe(III)-SOM from 18 (in the unamended soil) to

31-35%.

In conclusion, we provide evidence that size fractionation coupled with Fe speciation allows new insights about Fe mediated SOM stabilization in an agricultural soil subjected to organic amendments. Irrespective of the type of amendment, the FSi+Cl fraction accounted for most of the organic C ($53.5\pm5.7\%$) and total N ($67.6\pm3.2\%$) in comparison to the FSa fraction ($9.5\pm0.8\%$ and $3.5\pm0.5\%$, respectively). LCF and WT results of the EXAFS data show that FSa and FSi+Cl fractions consist of different proportions of ferrihydrite and Fe(III)-SOM. In the FSa fraction, the addition of the organic amendments resulted in an increase of Fe-SOM complexes (31-35%) compared to the unamended soil (18%) and a decrease of ferrihydrite (28-29% vs. 44% in the unamended soil). By contrast, in the amended FSi+Cl fractions, the freshly added organic matter led to negligible changes in percent ferrihydrite. Therefore, regardless of the amendment type (biochar or compost), the addition of exogenous organic matter to soil increases the capability of the coarser, FSa fraction to stabilize organic C.

Environmental implications and future perspectives

The FSa is supposed to play a secondary role for organic C partitioning in soils compared to the finer clay, due to its low surface area and cation exchange capacity. Our data clearly show that the addition of organic matter as an amendment significantly increased the Fe(III)-SOM in this coarser fraction; actually, the proportion of Fe(III)-SOM is amended vs. unamended soils was much higher in the FSa fraction compared to the FSi+Cl. This finding, while confirming that texture plays a role in soil organic C sequestration, underlines that the sand fraction may be an overlooked parameter in investigating organic matter accumulation in agricultural soils. Improving our mechanistic understanding of the role of the different soil size fractions in SOM stabilization is key to improve Earth

system models, as well as to design strategies to build up OM in agricultural soils. These strategies may not only sequester C in soils and help fight climate change, but are also essential to enhance other soil functions and services needed to achieve sustainable goals related to food, water, and biodiversity.

Future works will emphasize the possibility of ferrihydrite transformation dynamics and SOM accrual after amendments in FSa vs. FSi+Cl over time (e.g., years after the application of the organic amendments). Moreover, due to the experimental limitation on the number of samples that can be analyzed by EXAFS analysis, one must be cautious in over-interpretation of the spectroscopic results, especially considering that differences in organic ligands and mineralogy, typical of each single soil, could affect Fe(III)-SOM formation and environmental fate.^{58,59}

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Coupling EXAFS and selective extraction methods may provide additional, useful information to help addressing the specific Fe pools that have also formed. This can further explain the role played by SOM in the hindrance of Fe hydrolysis or dissolution, allowing us to more appropriately model our natural field conditions and potential of SOC build up.

Supporting Information

The Supporting Information is available free of charge on ...

Acknowledgements

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Figure captions

Figure 1.

Results for the 3-component LCF performed on the k^3 -weighted Fe K-edge EXAFS data, of fine sand (FSa) and fine silt and clay (FSi+Cl) pools of agricultural soils either unfertilized (UN), amended with biochar (BC), municipal solid waste compost (MC) and with both of them (BC+MC). Results are expressed in percentage of the fitting components corresponding to experimental spectra of model compounds reported in O'Day et al.⁴⁵: chlorite, ferrihydrite and Fe(III)-citrate (see also Table S4).

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Figure 2.

k^3 -weighted spectra of fine silt and clay (FSi+Cl) and fine sand (FSa) fractions of agricultural soils either unamended (UN), amended with biochar (BC), municipal solid waste compost (MC) and with both of them (BC+MC). Solid lines indicate the sample data, whereas dotted lines represent the fit. R-factors are also displayed.

Figure 3.

High resolution WT plots of standards displaying the second and third coordination shells ($\eta = 8$, $\sigma = 1$). Data are plotted as a function of k ($\text{\AA}^{-1}$) on the x axis and R ($\text{\AA}$) on the y axis in the range 2.0-4.0 ($\text{\AA}$).

Figure 4.

High resolution WT plots of fine silt and clay (FSi+Cl) and fine sand (FSa) fractions either unamended (UN), amended with biochar (BC), municipal solid waste compost (MC) and with both of them (BC+MC), displaying the second and third coordination

816 shells ($\eta = 8$, $\sigma = 1$): a) UN FSi+Cl; b) BC FSi+Cl; c) MC FSi+Cl; d) BC+MC FSi+Cl; e)
817 UN FSa; f) BC FSa; g) MC FSa; h) BC+MC FSa. Data are plotted as a function of k ($\text{\AA}^{-1}$)
818 on the x-axis and R ($\text{\AA}$) on the y-axis in the range 2.2-4.0 ($\text{\AA}$).

Table 1. Main mineral components of agricultural soils either unamended (UN), amended with biochar (BC), municipal solid waste compost (MC) and with both of them (BC+MC). Related XRD diffractograms are reported in Figure S1.

Ref. Code	Compound Name	UN FSa	MC FSa	BC+MC FSa	BC FSa
<i>SemiQuant [%]</i>					
00-029-0712	Ferrihydrite, syn	-	-	2	2
01-076-1819 / 01-083-1939	Albite low / Andesine	12	9	7	12
01-080-0885	Kaolinite	1	1	1	-
01-083-1762	Calcite	1	3	1	1
01-083-1766	Dolomite	1	-	1	2
01-084-1455	Microcline Pellotsalo - K-rich phase	14	14	8	11
01-085-0796	Quartz	68	69	76	67
01-085-1356	Chlorite Fe	1	1	1	1
01-086-1385	Muscovite 2M1	2	3	3	4
<i>SemiQuant [%]</i>					
		UN FSi+Cl	MC FSi+Cl	BC+MC FSi+Cl	BC FSi+Cl
00-029-0712	Ferrihydrite, syn	n.q.	n.q.	n.q.	n.q.
01-076-1819	Albite low	10	2	12	15
01-080-0885	Kaolinite	3	3	4	4
01-082-1873	Palygorskite	2	2	2	3
01-083-1762	Calcite	6	6	6	5
01-083-1766	Dolomite	2	2	2	4
01-084-1455	Microcline Pellotsalo - K-rich phase	15	16	16	15
01-085-0796	Quartz	48	58	43	34
01-085-1356	Chlorite Fe	3	3	4	5
01-086-1385	Muscovite 2M1	11	8	11	15

n.q. = not quantifiable

ha eliminato: Table 1. Total organic C content, total N, C/N ratio and Fe concentration (mean $\pm$ pooled standard error) of agricultural soils either unamended (UN), amended with biochar (BC), municipal solid waste compost (MC) and with both of them (BC+MC).¶

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ha eliminato: 2

Table 2. Organic C and N concentrations of fine sand (FSa) and fine silt and clay (FSi+Cl) pools of agricultural soils either unfertilized (UN), amended with biochar (BC), municipal solid waste compost (MC) and with both of them (BC+MC).

	FSa		FSi+Cl	
	Total organic C (g kg ⁻¹)	Total N (g kg ⁻¹)	Total organic C (g kg ⁻¹)	Total N (g kg ⁻¹)
UN	3.5	0.26	18.1	1.9
BC	8.2	0.33	25.1	2.1
MC	4.6	0.37	20.8	2.2
BC+MC	9.1	0.41	28.2	2.3

ha eliminato: 3

ha eliminato: ·

ha eliminato: ·

ha eliminato: ·

ha eliminato: ·

Figure 1.

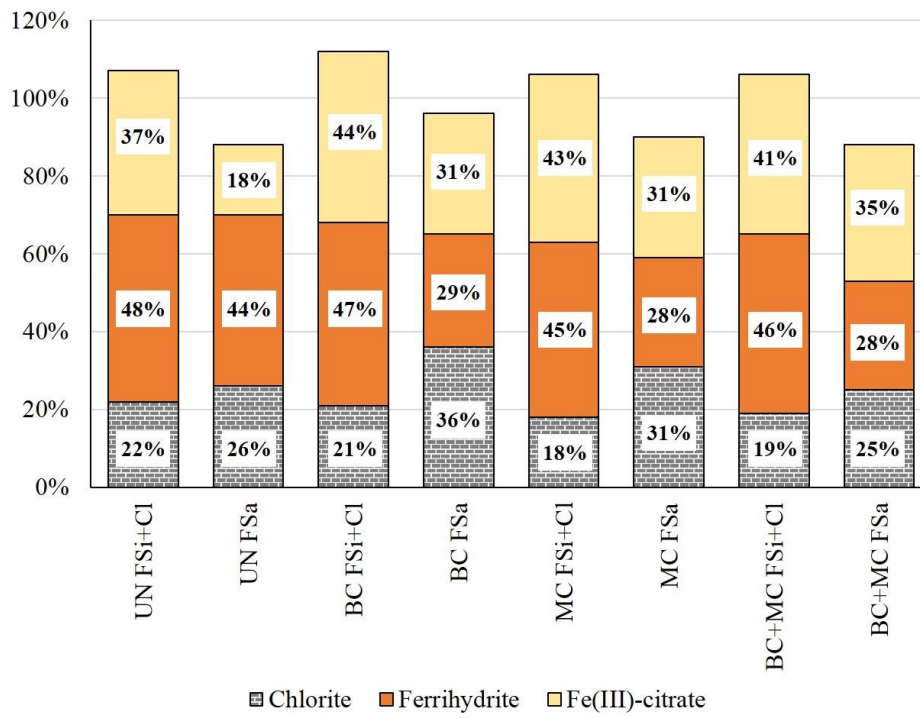


Figure 2.

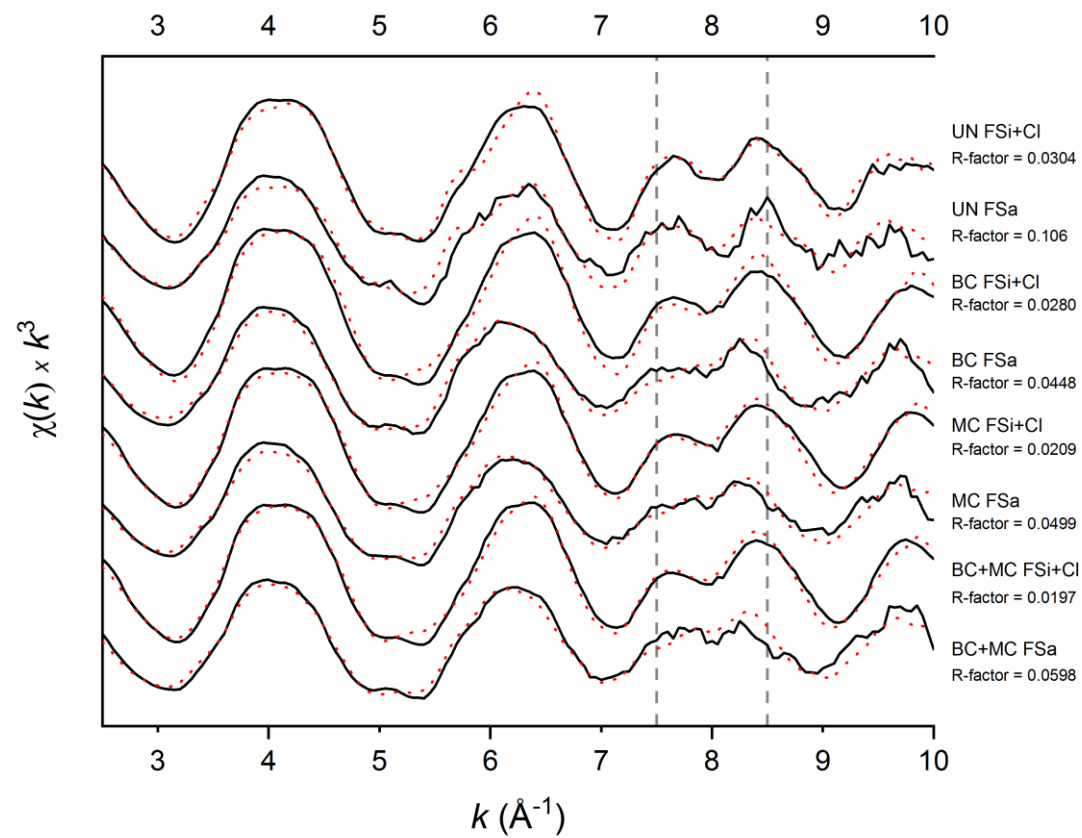


Figure 3.

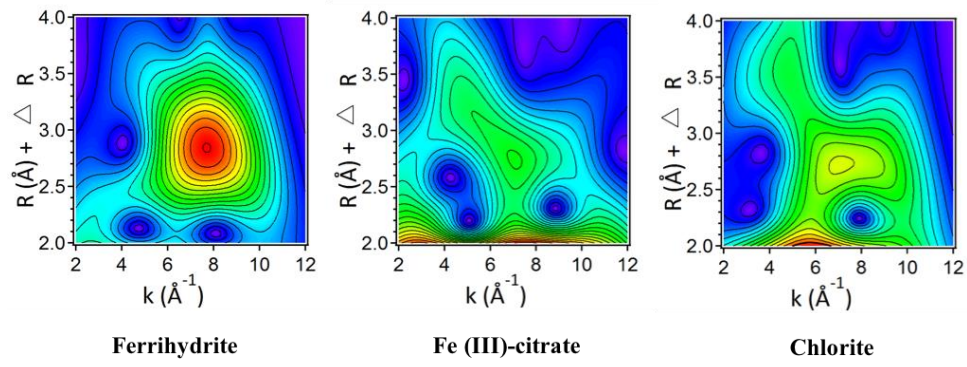


Figure 4.

