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Iron(III) fate after complexation with soil organic matter in fine silt and clay fractions: An EXAFS spectroscopic approach

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26 **Abstract**

27 Iron (Fe) speciation in soils is highly dependent on environmental conditions, mineralogy,  
28 and chemical interactions with soil organic matter (SOM). The fine silt and clay (FSi+Cl)  
29 particle size fraction of soils constitutes a primary organo-mineral fraction and contains  
30 SOM with long turnover time. In this study, the FSi+Cl particle size fractions isolated  
31 from a coniferous forest, a grassland, a technosol, and an agricultural soil were reacted  
32 with Fe(III) at pH 7. Unreacted and reacted samples were then investigated by means of  
33 extended X-ray absorption fine structure (EXAFS) spectroscopy. Statistical methods  
34 were used to determine goodness-of-fit parameters for linear combination fitting (LCF)  
35 and wavelet transformation (WT) of the Fe K-edge EXAFS data. WT separated spectral  
36 contributions from different backscattering atoms in higher coordination shells located at  
37 similar interatomic distances from the central absorbing Fe atom. LCF results paired with  
38 WT showed that the FSi+Cl particle size fractions consisted of a mixture of Fe  
39 phyllosilicates, Fe (hydr)oxides, and organically complexed Fe in different proportions.  
40 Our research revealed that after sorption experiments, in which Fe(III) was added to the  
41 system, increasing amounts of organic Fe(III)-SOM complexes were found in the solid  
42 phase of grassland and agricultural soils, whereas the precipitation of Fe(III) led to the  
43 preferential formation of ferrihydrite in the coniferous forest soil and in the technosol.  
44 Although the quantitative Fe-mediated organic carbon stabilization effect after Fe(III)  
45 addition is shown in this work, Fe speciation is not clearly related to SOM amount or  
46 quality (*i.e.*, carbon-to-nitrogen ratio). The variation of Fe chemical speciation among the  
47 soil fractions likely translates into differences in their environmental fate.

48

49 **Keywords:** physical fractionation; Fe speciation; linear combination fitting.

50 **Introduction**

51 With 3,000 Pg of carbon (C) (Köchy et al., 2015), soils represent the largest C stock on  
52 the Earth's surface and play a pivotal role in regulating the global C cycle (Blanco-Canqui  
53 and Lal, 2004; Lal, 2004; Lehmann et al., 2007). Understanding soil organic C (SOC)  
54 dynamics and how it responds to soil management is extremely important especially  
55 under the current climate change scenario.

56 The formation of protective associations between minerals and soil organic matter  
57 (SOM) is important to prevent further release of carbon dioxide (CO<sub>2</sub>) from soils into the  
58 atmosphere. The finer fraction of soil constituents (*i.e.*, silt and clay) is important because  
59 it contains a mixture of mineral phases with high surface area (Feng et al., 2013; Barré et  
60 al., 2014), which is an important driver of C sequestration potential in soils (Kögel-  
61 Knabner et al., 2008).

62 The fine silt and clay (FSi+Cl) soil fraction is characterized by the presence of multiple  
63 iron (Fe) mineral phases, ranging from Fe(II)/Fe(III) phyllosilicates to poorly or highly  
64 crystalline Fe-oxides, hydroxides, and oxyhydroxides. Fe-(hydr)oxide minerals are  
65 ubiquitous in soils (Schwertmann, 1991) and represent an important phase for the  
66 stabilization of SOC (Jambor and Dutrizac, 1998; Eusterhues et al., 2005; Lalonde et al.,  
67 2012). Fe-SOM complexation, which is affected by the nature of SOM (Rose et al., 1998),  
68 influences long-term C sequestration and can modify the reactivity and structure of Fe  
69 (hydr)oxides (Eusterhues et al., 2008, 2014). In particular, Fe oxide crystal structure is  
70 affected by coprecipitation with humic substances, which inhibits their transformation  
71 into more stable forms (*e.g.*, ferrihydrite to goethite) (Schwertmann, 1966; Schwertmann  
72 et al., 2005). Although calcium (Ca) is believed to be the main SOM stabilizer cation in  
73 the calcareous soils typical of the Mediterranean area (Giannetta et al., 2019a), Fe  
74 oxyhydroxides may be also important in the long-term stabilization of SOM (Mikutta et

75 al., 2006; Kögel-Knabner et al., 2008). Thus, improving our understanding about the  
76 molecular structure and hydrolysis of Fe species formed in association with SOM and the  
77 stabilization mechanisms of SOM-Fe coprecipitates is fundamental to predict the  
78 response of soil Fe and organic C to changes in global climate and land use (Post and  
79 Kwon, 2000).

80 Recently, state-of-the-art synchrotron-based techniques, such as Extended X-ray  
81 Absorption Fine Structure (EXAFS) spectroscopy, have been solely used for the  
82 speciation of model metal (hydr)oxide systems (pure Fe (hydr)oxides) and Fe(III)  
83 complexation with different types of SOM, including dissolved organic matter (DOM)  
84 (Nierop et al., 2002; Eusterhues et al., 2008, 2011; Henneberry et al., 2012; Chen et al.,  
85 2014), peats (Karlsson and Persson, 2010), humic substances (Mikutta and Kretzschmar,  
86 2011; Shimizu et al., 2013), and small organic acids (Mikutta, 2011; Yang et al., 2016),  
87 in addition to bulk soils and waters (Sundman et al., 2014). In contrast, the chemical  
88 interactions between Fe species and physically-fractionated SOM pools have not been  
89 deeply investigated, and it is virtually unknown how Fe-mediated stabilization of SOM  
90 in natural rather than model systems can control the persistence of organic C as a solid  
91 phase in the soil. Thus, there is a gap between these modelled systems and natural  
92 systems, which include soils from a variety of land uses.

93 As an additional step to previous sorption/desorption experiments (Giannetta et al.,  
94 2019b), here we investigated, by solid phase Fe speciation, the effects of added Fe on Fe  
95 minerals and SOM contained in the FSi+Cl fraction of soils with different land use. The  
96 main objective of this study was to test the formation of Fe(III)-SOM complexes and  
97 Fe(III) precipitates as Fe(III) oxides in FSi+Cl fractions subjected to coprecipitation  
98 experiments. These products were determined via Fe speciation analysis of Fe K-edge  
99 EXAFS spectra. This important step in the research on SOM-Fe mineral complexation is

100 challenging because of the heterogeneity of SOM and Fe minerals naturally present in  
101 soils.

102

## 103 **2. Materials and methods**

### 104 *2.1. Soil samples*

105 The soil samples were collected from a coniferous forest (CF), a grassland (GL), a  
106 technosol (TS) and an agricultural field (AG). Mean annual temperature of sampling sites  
107 ranged from 9.7 and 14.8 °C, whereas mean annual precipitation ranged between 440 and  
108 980 mm. The coniferous forest consisted mainly of *Pinus nigra* woodlands occurring at  
109 630 m a.s.l., whereas the pasture, grown around 1000 m a.s.l., was dominated by meso-  
110 to xerophilous species (*e.g.*, *Bromus erectus*). The technosol consisted of a dump  
111 reclaimed in 2000, while the AG soil was an experimental field located at 530 m a.s.l.  
112 The main physical and chemical properties of soils included in this study are described  
113 elsewhere (Plaza et al., 2016; Giannetta et al., 2018, 2019b) and summarized in Table 1.

114

### 115 *2.2. Physical fractionation*

116 A physical size fractionation by ultrasonic dispersion and wet sieving was performed,  
117 allowing for separation of particles into four different size fractions: coarse sand (CSa:  
118 2000-200 µm diameter), fine sand (FSa: 200-50 µm), coarse silt (CSi: 50-20 µm) and fine  
119 silt and clay (FSi+Cl: <20 µm) (Lopez-Sangil and Rovira, 2013). 15 g of each sample  
120 were put into 50 mL vials and filled with deionized water to 3/4 of their volume. Samples  
121 were then subjected to vertical agitation (20 rpm) for 60 minutes and to ultrasonic  
122 dispersion of the soil particles at an energy input of 1020 J mL<sup>-1</sup> for 10 minutes using a  
123 Branson 45 sonifier. The derived suspensions were then wet-sieved through a set of three  
124 sieves (200 µm, 50 µm and 20 µm mesh) splitting particles into four different size

125 fractions. The fractions retained by sieves (CSa, FSa, CSi) were quantitatively transferred  
126 to pre-weighted vials and dried at 60 °C to constant weight. The particles passing through  
127 the last sieve (<20 µm: FSi+Cl) were brought to *ca.* 1 L and left to stand refrigerated for  
128 2 days; overlying water was then carefully siphoned off and discarded. The sediment was  
129 transferred into 250 mL polypropylene vials and centrifuged for 15 min at 2500 g and  
130 then dried at 60 °C. In this research, we used exclusively the FSi+Cl fractions, which are  
131 the fractions with the highest specific surface area and expected to be the most active with  
132 respect to organic C and Fe(III) sorption.

133

### 134 *2.3. Characterization of FSi+Cl fractions*

135 X-ray powder diffraction was conducted using a Philips X'Pert diffractometer with Cu  
136 K $\alpha$  radiation and a scan rate of 0.02° 2 $\theta$  s<sup>-1</sup>. XRD patterns were collected in the 3–70° 2 $\theta$   
137 range. The mineral composition was determined by comparison with the reference  
138 intensity ratio (RIR) values in the powder diffraction database of the International Centre  
139 for Diffraction Data (Chung, 1974a,b, 1975), using the software X'Pert HighScore 2.2e  
140 (Malvern Panalytical, Malvern, UK).

141 Total organic C and N contents were determined by dry combustion using a Thermo Flash  
142 2000 NC Soil Analyzer. Each sample was ground with a ball mill and subjected to acid  
143 fumigation before analysis to remove carbonates (Harris et al., 2001). Total Fe content  
144 was determined by Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-  
145 OES) after digestion with nitric and perchloric acid.

146

### 147 *2.4. Sorption experiments*

148 Fe(III)-SOM complexation was conducted following the methods proposed by Karlsson  
149 and Persson (2010) and Mikutta and Kretzschmar (2011) with slight modifications. In

150 particular, 200 mg of each fraction was weighted into a 50 mL polypropylene vial.  
151 Acidified Fe(III) nitrate stock solution (12.15 mM) was added to each sample to reach a  
152 loading of 200 mg Fe /g C. The pH of the slurry was immediately raised to 7 and  
153 monitored during the next 24 hours and adjusted to  $7\pm 0.5$  with 0.1 M KOH. The  
154 suspensions were shaken at 60 rpm at room temperature for 24 hours and then centrifuged  
155 at 10,000 rpm for 20 minutes. The supernatant was removed and filtered through a 0.2  
156  $\mu\text{m}$  polysulfone membrane filter and acidified with 2%  $\text{HNO}_3$  (trace metal grade).  
157 Filtrates were analyzed for both dissolved organic C (DOC), using a Vario TOC cube  
158 analyzer, and Fe concentration, by ICP-MS. These sorption experiments were conducted  
159 in duplicate at room temperature and the results described in Giannetta et al. (2019b).

160

#### 161 *2.5. Iron K-edge Extended X-ray Absorption Fine Structure (EXAFS) spectroscopy*

162 The speciation of Fe in the solid phase before and after the reaction from our previous  
163 sorption study (Giannetta et al., 2019b) was analyzed by X-ray absorption spectroscopy  
164 (XAS), which generally has a beam size of ca.  $10\text{ mm}^2$ . This spot size is thousands of  
165 times larger than other techniques commonly used to study SOM functional groups in a  
166 sample (Stuckey et al., 2017). XAS analyses of reacted and unreacted samples were  
167 conducted at Beamlines 4-1 and 4-3 at the Stanford Synchrotron Radiation Lightsource  
168 (SSRL) in Menlo Park (CA, USA). Fe K-edge EXAFS were collected in transmission  
169 mode. X-ray energy was maintained by a Si (222) crystal monochromator at 4-1 and Si  
170 (111) at 4-3. The monochromator was detuned 50% to reduce higher order harmonics.  
171 Ground samples (15-30 mg) were prepared as pressed pellets between Kapton® tape. Two  
172 to six replicate scans were collected in order to obtain satisfactory signal to noise ratios,  
173 with energy calibrated against an Fe (0) foil to the inflection point of the first derivative  
174 (7112 eV).



175 Statistical methods have been used to determine goodness-of-fit parameters for linear  
176 combination fitting (LCF) and wavelet transformation (WT). WT is useful to separate  
177 contributions from different backscattering atoms in higher coordination shells located at  
178 similar interatomic distances from the central absorbing Fe atom. A detailed description  
179 of the methods used is reported in the Supporting Information.

180

### 181 **3. Results and Discussion**

#### 182 *3.1. Mineralogy and elemental composition of the FSi+Cl fractions*

183 The FSi+Cl fractions isolated from CF and GL show a similar composition in terms of  
184 both main mineral phases and relative percentages. In particular, both FSi+Cl fractions  
185 consist mainly of quartz (34-36%), calcite (19%), illite (18-19%) and microcline (10%)  
186 (Table 2). The FSi+Cl fraction isolated from TS is similar to the previous ones but shows  
187 a higher calcite content (30%) and slightly lower contents of microcline (4%) and quartz  
188 (Table 2). In contrast, the FSi+Cl fraction isolated from AG shows significant differences  
189 in terms of mineral composition, being characterized by significantly higher contents of  
190 quartz (44%), kaolinite (8%) and chlorite (6%) and a lower content of calcite (4%);  
191 moreover, muscovite (12%) occurs exclusively in this sample, whereas no  
192 montmorillonite and illite are detected (Table 2).

193 Organic C and total N contents of the examined fractions is shown in Table 3. Organic C  
194 content ranges from 0.7 to 8.5 g kg<sup>-1</sup>, whereas total N varies between 0.08 and 0.76 g kg<sup>-1</sup>.  
195 <sup>1</sup>. In all the cases, the lowest and the highest concentration values are found in the FSi+Cl  
196 fraction from TS and GL, respectively. The corresponding C/N ratios, ranging from 8 to  
197 13, could point to an organic matter fraction of different origin.

198

#### 199 *Quantitative Fe speciation in FSi+Cl fractions*

200 LCF results are reported in Table 4, revealing the standards that best reconstruct the  
201 sample data. Although Fe EXAFS provides a good estimation of the relative contribution  
202 from different Fe phases (*e.g.*, discrimination of Fe oxyhydroxides and Fe-organic  
203 complexes), it is well reported that differentiating among specific Fe(III) oxyhydroxides  
204 (*e.g.*, ferrihydrite, goethite and hematite) can be difficult (O'Day et al., 2004; Prietzel et  
205 al., 2007). Application of the F-test (Hamilton test) and use of the scree plot (Figure S1),  
206 as well as Principal Components Analysis (PCA) and Target Transformation (TT), were  
207 employed to determine the appropriate standards for LCF since the increase and decrease  
208 of only one standard can heavily influence the calculated percentage of each component  
209 (Siebecker et al., 2017). TT values are described in Table S1.

210 Thus, three standards were used for LCF: (1) Fe in phyllosilicates bound in the  
211 octahedral layer; (2) ferrihydrite was selected to represent pedogenic Fe(III)-minerals;  
212 and (3) Fe(III)-citrate as a model compound for organically bound Fe (O'Day et al.,  
213 2004). Fe(III)-citrate was chosen to represent Fe complexed with low-molecular weight  
214 organic acids (LMWOA), being one of the major constituents in microbial metabolites  
215 and root exudates (Yang et al., 2016) and assuming that organically bound Fe is mainly  
216 bound to carboxylate groups of deprotonated LMWOA (Pohlman and McColl, 1988;  
217 Prietzel et al., 2007). Moreover, LMWOA represent a common soil constituent, are partly  
218 redox-active, and their interaction with Fe(III)-hydroxides is comparable to that with  
219 SOM (Evanko and Dzombak, 1998).

220 The LCF of Fe EXAFS data of unreacted soils revealed that the ferrihydrite  
221 component varied from 23% in CF to 48% in GL, TS and AG, while Fe(III)-SOM  
222 complexes, reported as Fe(III)-citrate, ranged from 18% in GL to 41% in CF (Table 3).  
223 After the addition and precipitation of Fe(III), a small increase in ferrihydrite percentage  
224 is noticeable in CF (from 23 to 30%) and TS (from 47 to 52%), whereas in GL and AG a

225 reduction in ferrihydrite percentage is observed. On the other hand, the Fe(III)-citrate  
226 percentage increased in GL (from 18 to 23%) and AG (from 37 to 46%), and decreases  
227 in CF (from 41 to 35%) and TS (from 33 to 28%) (Table 4).

228 Fe(III)-silicates are represented by illite (39%) in CF, smectite (31%) in GL, and  
229 chlorite in TS and AG (26% and 22%, respectively). The illite reference sample contains  
230 7.32 wt % Fe<sub>2</sub>O<sub>3</sub> and 0.55 wt % FeO substituted in the octahedral layer for Al. Smectite  
231 is a low-Fe standard with 1.42% Fe<sub>2</sub>O<sub>3</sub> and 0.88 wt % FeO that also contains Al and Mg  
232 in the octahedral layer (O'Day et al., 2004).

233

### 234 3.2. Qualitative Fe speciation in FSi+Cl fractions

235 LCF results are also shown in Figure 1, where the fit of each sample is represented as  
236 dotted lines and can be compared to the EXAFS spectra of the standards (Figure S2). Fe  
237 K-edge EXAFS spectra of unreacted samples show similar features at ~4.0 Å<sup>-1</sup> (peak 1),  
238 ~6.5 Å<sup>-1</sup> (peak 2), ~7.5 Å<sup>-1</sup> (peak 3), and ~8.5 Å<sup>-1</sup> (peak 4). The peak at 4.0 Å<sup>-1</sup> remained  
239 unchanged after reaction with Fe(III). Conversely, the peak at 7.5 Å<sup>-1</sup>, characteristic of  
240 ferrihydrite (Mikutta, 2011), becomes less evident in GL and AG.

241 The applicability of LCF to quantify different Fe phases in heterogeneous materials  
242 such as soils and different particle size fractions still represents an obstacle because often  
243 natural constituents can be only partially represented by Fe minerals synthesized in the  
244 laboratory (Prietz et al., 2007). For this reason, the LCF values often do not sum to  
245 100%. Conversely, in model systems, such synthetic ferrihydrite, shell fitting analysis of  
246 the second-shell can be carried out because the systems are simplified. The Fe–C and Fe–  
247 Fe distances in those model systems describe the coordination modes of the organic Fe  
248 complexes and the polymeric Fe (hydr)oxides, respectively. Gustafsson et al. (2007)  
249 reported that Fe(III) in organic soils (pH 4) occurred either as Fe (hydr)oxides or

250 organically complexed likely as a mixture of di- and trinuclear  $(\text{O}_5\text{Fe})_2\text{O}$  and  $(\text{O}_5\text{Fe})_3\text{O}$   
251 complexes. Rose et al. (1998) determined the speciation of Fe in natural organic matter  
252 (NOM) from freshwaters (pH 5.5–7.5) and found that Fe was poorly polymerized due to  
253 complexation with NOM. In our study, Fe complexation with NOM is analogous to the  
254 Fe(III)-citrate standard.

255

### 256 *3.3. Correlation between the distribution of organic C and total N and Fe speciation in* 257 *FSi+Cl fractions*

258 There is a positive and significant correlation between the organic C and Fe content  
259 ( $p < 0.05$ ), as well as between total N and Fe content ( $p < 0.05$ ) (Table S2). In particular,  
260 GL and CF exhibit the highest contents of both organic C (8.5 and 5.4%, respectively)  
261 and Fe (10.3 and 9%, respectively) (Table 3) and had the lowest amount of C released  
262 into solution after the reaction at 1.8 and 1.6%, respectively (Giannetta et al., 2019b).  
263 However, there is no correlation between the Fe species and organic C and total N. The  
264 lack of significant correlation between organic C content in each soil and the Fe species  
265 means that (a) the relationship between Fe and C are either weaker than expected, (b)  
266 organic C content does not drive Fe speciation in these specific samples, or (c) a larger  
267 sample size is needed.

268 CF is characterized by the highest Fe(III)-SOM percentage (41%) and the lowest  
269 ferrihydrite (23%), and GL is characterized by the lowest Fe(III)-SOM percentage (18%)  
270 and the highest ferrihydrite (48%). After the reaction in CF, the precipitation of Fe(III)  
271 seems to lead to the preferential formation of ferrihydrite. This finding is in agreement  
272 with previous results reported in Giannetta et al. (2019b), who observed an increase in  
273 the carbohydrates band relative intensity in attenuated total reflectance Fourier transform  
274 infrared (ATR-FTIR) spectra after the reaction with Fe(III). This was ascribed to the

275 possible formation of inner-sphere surface complexes between the carbohydrate OH  
276 functional groups and the Fe atoms on the surface of ferrihydrite (*i.e.*, the formation of  
277 surface complexes between labile C sources and newly formed ferrihydrite) (Eusterhues  
278 et al., 2014, 2011). It has been shown that ferrihydrite particles can be stabilized by an  
279 encapsulating layer of polysaccharides (Karlsson and Persson, 2012). In the unreacted  
280 fraction of GL, the dominant component of Fe was present as ferrihydrite, and after the  
281 reaction a slight increase in the Fe citrate component was found. Giannetta et al. (2018)  
282 hypothesized that SOM in TS is enriched in newly formed organic molecules (*e.g.*, sugars,  
283 amino acids, proteins), mainly resulting from microbial transformation rather than in  
284 highly degraded litter inputs. These labile compounds can associate with mineral surfaces  
285 (*e.g.*, Fe(III) that precipitates as Fe(III) hydroxides) in the same way described for CF.  
286 For the AG soil, previously reported ATR-FTIR results demonstrated that small  
287 differences in carbohydrates peaks can be indicative of labile SOM associated with  
288 minerals as well as the formation of Fe-SOM complexes (Giannetta et al., 2019b); this  
289 was also discussed by Karlsson and Persson (2012) and is in agreement with the increase  
290 in Fe(III)-SOM in AG and the decrease in ferrihydrite.

291 Organic functional groups (ligands) found in SOM can also impact Fe mineral  
292 cycling. Ligand-promoted dissolution at neutral pH can have a large impact on Fe  
293 speciation in soils because they affect ferrihydrite formation and can exert a strong  
294 influence on its structure (Mikutta et al., 2008). Mikutta et al. (2010) reported that organic  
295 chelates such as hydroxybenzoic acids control the kinetics of ferrihydrite formation via  
296 Fe(III) complexation, thereby effectively lowering the solution saturation state of  
297 inorganic Fe(III) species, leaving less Fe(III) available for ferrihydrite precipitation. This  
298 has been demonstrated in laboratory systems and likely also occurs in soils.

299

300 3.4. Wavelet analysis of higher coordination shells

301 As in EXAFS studies on Fe associated with SOM, the application of traditional EXAFS  
302 analyses (*e.g.*, shell fitting of the Fourier transform; FT) can be challenging to identify  
303 specific species in heterogeneous systems such as soils; thus we also employed WT to aid  
304 in discriminating scattering elements. This challenge is due to the need to separate  
305 contributions from different backscattering atoms in coordination shells at similar  
306 interatomic distances from the central absorbing Fe atom. WT can provide useful  
307 information to simultaneously resolve data in both k-space ( $\text{\AA}^{-1}$ ) and interatomic distance  
308 (R-space,  $\text{\AA}$ ), helping to differentiate lighter backscattering elements when they are in the  
309 same coordination shell from the central adsorbing atom (*e.g.*, Fe *vs.* C) (Funke et al.,  
310 2005, 2007). WT represents an analogue to the FT and reveals the energies at which the  
311 backscattering components are most significant in addition to the distances of the  
312 backscattering atoms. WT *k*-R maps are helpful in improving the fitting model and  
313 understanding local atomic structure (Xia et al., 2018). In a parallel experiment (Giannetta  
314 et al., submitted) we applied this approach, pairing LCF and WT to illustrate the role  
315 played by organic inputs (*i.e.*, biochar and compost) to determine Fe speciation and  
316 distribution in an agricultural soil. Here, WT analysis has been used to qualitatively test  
317 for the presence of Fe backscatterers in the second coordination shell of the Fe K-edge  
318 EXAFS data. We calculated the Morlet wavelet transforms of  $k^3$ -weighted Fe K-edge  
319 EXAFS spectra of the samples over a  $R + \Delta R$ -range of 2.2-4.0  $\text{\AA}$  (*i.e.*, the second  
320 coordination shell) and compared the resulting wavelet plots with those of Fe(III)  
321 reference compounds (ferrihydrite, Fe(III)-citrate, illite, smectite, and chlorite).

322 Figure 2 contains the WT plots of the standards, whereas Figure 3 displays the WT  
323 plots of the reacted and non-reacted samples. In the ferrihydrite plot, the Fe shell  
324 contributes a strong feature at 7-8  $\text{\AA}^{-1}$  and 2.75  $\text{\AA}$ , corresponding to the second peak in

325 the FT (Daugherty et al., 2017). In Fe(III)-SOM spectra (Fe-citrate) there is no Fe  
326 backscattering, but features at 2.0–2.5 Å indicate back-scattering from lighter atoms.  
327 These features are in agreement with single and multiple backscattering from C/O  
328 (Persson and Axe, 2005; Karlsson et al., 2008; Karlsson and Persson, 2010; Daugherty et  
329 al., 2017). Moreover, these features appear at lower energies (3–4 Å<sup>-1</sup>) than those of Fe.  
330 The illite plot presents a strong feature at 8 Å<sup>-1</sup> and 2.8 Å (O’Day et al., 2004), similar to  
331 the ferrihydrite. This peak, however, is very weak in the chlorite and smectite WT plots,  
332 as compared to illite and ferrihydrite. The illite sample has 7.32 wt% Fe<sub>2</sub>O<sub>3</sub> and 0.55 wt%  
333 FeO, presumably substituted in the octahedral layer for Al. The smectite is a low Fe  
334 variety with 1.42 wt% Fe<sub>2</sub>O<sub>3</sub> and 0.08 wt% FeO, that contains mostly Al and Mg in the  
335 octahedral layer (O’Day et al., 2004). Thus, due to the lower total Fe content, the second-  
336 neighbour backscattering is lower in amplitude for smectite rather than for illite. The lack  
337 of an intense maximum in WT plots of the smectite and chlorite standards is related to  
338 their less intense second shell peak in the FT (Figure S3). In general, the first two major  
339 shells in phyllosilicate clay minerals can be described by three interatomic backscattering  
340 paths: (1) the first-neighbour O, (2) second-neighbour octahedral Fe, Al, or Mg; and (3)  
341 tetrahedral Si (Figures 2 and S3). In many Fe oxyhydroxides and phyllosilicates, the Fe-  
342 Fe neighbour distances overlap at 2.75 Å, thus the analysis of the second coordination  
343 shell is particularly complicated.

344 WT of the EXAFS data from the samples and the FT spectra in Figure S4 showed  
345 that the features ascribed to light backscatterers at low k-values and by heavier atoms at  
346 higher k varied among the unreacted and Fe(III) reacted samples (Figure 3). The WT plot  
347 of the unreacted CF sample indicates a Fe-Fe shell contribution similar to both ferrihydrite  
348 and illite. A contribution from Fe(III)-citrate at 2.2 Å and 2–4 Å<sup>-1</sup> is also evident,  
349 supporting the LCF results (41% of Fe(III)-citrate). The plot of the CF reacted sample

350 displays features typical of ferrihydrite, which increased from 23% to 30% according to  
351 the LCF result. The Fe-Fe scattering feature in unreacted GL appears at the distance in  
352 both k- and R-space ascribed to ferrihydrite. According to LCF results, GL contained the  
353 lowest Fe-citrate percentage, thus no features from C backscattering are evident in the  
354 WT plot. At  $\eta=8$ , differences between unreacted and reacted samples are not evident.  
355 However, at  $\eta=6$  (Figure S6b), in the reacted GL sample, a feature at  $2.2 \text{ \AA}$  appears and  
356 it is related to C backscattering. The TS WT plot shows a clear indication of heavy  
357 element back-scattering atoms. Noticeable differences are not seen in the reacted plot. In  
358 AG, the presence of 37% of Fe(III)-SOM from LCF is supported by the presence of the  
359 Fe(III)-SOM feature at  $2.2 \text{ \AA}$  and  $2-4 \text{ \AA}^{-1}$ . After the reaction this feature increases, as the  
360 Fe(III)-citrate increases up to 46%, in agreement with the results reported in LCF.  
361 Moreover, the same peak at  $2.2 \text{ \AA}$  is increasing in the FT spectrum displayed in Figure  
362 S4.

363 When modifying  $\eta$  from 9 to 4 for both the standards (Figure S5) and the samples  
364 (Figure S6a-d), at 6, the maximum starts to separate into separate peaks, indicating the  
365 splitting of the major component into two minor components.

366 The second shell of phyllosilicate clay minerals results from the scattering path from  
367 different elements (as discussed above in this section); and this separation seen in the WT  
368 plots can be ascribed to the second-neighbour octahedral Fe, Al, or Mg, and tetrahedral  
369 Si, the splitting in two different components being a common feature of illite and smectite  
370 (Figures S5 and S6). In AG the splitting into separate peaks at  $\eta=6$  is not as strong (Figure  
371 S6d), as in the other samples, being AG characterized by low chlorite percentages, both  
372 in unreacted (22%) and reacted (14%) samples.

373 These results also highlight that WT interpretation is challenging due to the overlapping  
374 of the Fe-Fe neighbour distances in many Fe oxyhydroxides and phyllosilicates.



375 However, the WT results indicated that the second shell of reacted samples consisted of  
376 either lighter scattering elements such as C/O atoms and/or heavier scattering elements,  
377 such as Fe. Our data indicate that the formation of Fe(III)-SOM complexes in soils with  
378 more labile organic C may perhaps suppress the hydrolysis and polymerization of Fe(III).  
379 These complexes have different reactivity as compared to Fe(III) in (hydr)oxide phases  
380 or other Fe(III)-bearing minerals. Lastly, the WT results support the LCF analysis, which  
381 indicated that EXAFS spectra of the soil samples could be described as a mixture of Fe-  
382 bearing clay minerals, Fe (hydr)oxides and organically complexed Fe.

383

#### 384 **4. Conclusions**

385 Here we identify how SOM can influence Fe(III) speciation by adding Fe(III) to the silt  
386 and clay fraction of different soils, and we demonstrate how both Fe(III)-SOM complexes  
387 and Fe(III) polymerization (*i.e.*, the formation of iron oxyhydroxides) can vary between  
388 each sample. Additionally, changes in SOM reactivity in these samples was previously  
389 identified, which has consequences for SOM stabilization in FSi+Cl fractions; for  
390 example, adding Fe(III) changed Fe speciation and decreased soluble DOC (Giannetta et  
391 al., 2019b). We underline the importance of pairing LCF with WT to resolve data in both  
392 k-space (energy) and interatomic distance (R-space), helping to differentiate lighter  
393 backscattering elements when they are in the same coordination shell from the central  
394 adsorbing Fe atom.

395 Batch sorption data from our past work (Giannetta et al., 2019b) have been used to  
396 probe the effect of different natural ecosystems on Fe(III) precipitation and complexation  
397 mechanisms. In a second step, here, both LCF and WT results indicated that the soil  
398 samples can be described as a mixture of Fe-bearing phyllosilicate clay minerals, Fe  
399 (hydr)oxides, and organically complexed Fe. Our research has revealed that after sorption

400 experiments in which Fe(III) was added to the system, increasing amounts of organic  
401 Fe(III)-SOM complexes in the solid phase of GL and AG soils were found; on the other  
402 hand, in the FSi+Cl fractions of the CF and TS soils, the precipitation of Fe(III) lead to  
403 the preferential formation of ferrihydrite. Finally, although the quantitative Fe-mediated  
404 organic C stabilization effect after Fe(III) addition was previously demonstrated  
405 (Giannetta et al., 2019b), the Fe speciation is not clearly related to SOM amount and C/N  
406 ratio.

407 In conclusion, the combination of Fe EXAFS with size-fractioned samples represents  
408 an important approach to study Fe dynamics in natural environments, as it helps unravel  
409 links among Fe originally present in the soil, formation mechanisms of different Fe  
410 species (either organically bound Fe or Fe-oxides), and the structures of those species.

411 Future investigations should focus on the influence of SOM on Fe speciation and  
412 complexation during changing environmental conditions (mainly redox potential and pH)  
413 and land use (*e.g.*, forest *vs.* agricultural soil). In particular, although the present study  
414 underlines different organic C sequestration potential among finer fractions isolated from  
415 soils under diverse land uses, a larger number of samples should be investigated in order  
416 to validate this hypothesis and avoid an over-interpretation of the spectroscopic results.  
417 In fact, the differences observed in our study in terms of Fe(III)-SOM formation could  
418 also be potentially related to the differences in organic ligands and mineralogy  
419 characterizing each of the studied soils, as both contribute to the binding energy of SOM  
420 (Newcomb et al., 2017).

421

#### 422 **Declaration of Competing Interest**

423 The authors declare that they have no known competing financial interests or personal  
424 relationships that could have appeared to influence the work reported in this paper.

425

426 **Appendix A. Supplementary data**

427 The following is Supplementary data to this article: ...

428

429

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438

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622 **Figure captions**

623

624 **Figure 1.**

625  $k^3$ -weighted EXAFS spectra of fine silt and clay (FSi+Cl) fractions from coniferous  
626 forest (CF), grassland (GL), technosol (TS) and agricultural (AG) soils before (U) and  
627 after (R) reaction with Fe(III). Solid lines indicate the sample data, whereas red dotted  
628 lines represent the LCF fit.

629

630 **Figure 2.**

631 High resolution WT plots of standards displaying the second coordination shell. Data are  
632 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  
633 ( $\text{\AA}$ ).

634

635 **Figure 3.**

636 High resolution WT plots of fine silt and clay (FSi+Cl) fractions from coniferous forest  
637 (CF), grassland (GL), technosol (TS) and agricultural (AG) soils before (U) and after (R)  
638 reaction with Fe(III) displaying the second shell. Data are plotted as a function of  $k$  ( $\text{\AA}^{-1}$ )  
639 on the x axis and  $R$  ( $\text{\AA}$ ) on the y axis in the range 2.2-4.0 ( $\text{\AA}$ ).

640 **Table 1.** Site details and main physical and chemical properties of the coniferous forest (CF), grassland (GL), technosols (TS) and agricultural  
 641 (AG) soils included in this study. Data are from Giannetta et al. (2018, 2019b).

Sample	CF	GL	TS	AG
<b>Site</b>	San Lorenzo (San Severino Marche, Italy)	Forcatura (Fiuminata, Italy)	Asola (Potenza Picena, Italy)	Arganda del Rey (Madrid, Spain)
<b>Coordinates</b>	13°14'03" E; 43°18'05" N	12°53'49" E; 43°08'51" N	13°14'49" E; 43°13'34" N	3°29'06" W; 40°18'58" N
<b>Depth (cm)</b>	0-25	0-15	0-20	0-15
<b>Soil Taxonomy</b>	Lithic Rendoll	Typic Haplustoll	-	Xerofluvent
<b>Land use</b>	Reforestation with <i>Pinus nigra</i> (dominant species); age: 60 years	Natural or semi-natural meadows and pastures	Dump reclaimed in 2000	Unamended, agricultural soil, planted with <i>Hordeum vulgare</i>
<b>pH</b>	7.9	7.6	8.5	8.4
<b>Sand (<math>g \cdot kg^{-1}</math>)</b>	527	631	142	339
<b>Silt (<math>g \cdot kg^{-1}</math>)</b>	212	202	452	471
<b>Clay (<math>g \cdot kg^{-1}</math>)</b>	261	167	406	190
<b>Organic C (<math>g \cdot kg^{-1}</math>)</b>	77.9	93.0	7.5	11.8
<b>Total N (<math>g \cdot kg^{-1}</math>)</b>	4.5	7.7	0.8	1.1
<b>C/N</b>	17.4	12	9	10.7
<b>Fe (<math>g \cdot kg^{-1}</math>)</b>	30.9	27.5	21.2	22.1

642

643 **Table 2.** Mineralogical composition of the fine silt plus clay (FSi+Cl) fraction isolated  
 644 from a coniferous forest soil (CF), a grassland soil (GL), a technosol (TS) and an  
 645 agricultural soil (AG).  
 646

PDF ref. number	Compound name	CF FSi+Cl	GL FSi+Cl	TS FSi+Cl	AG FSi+Cl
		<i>SemiQuant [%]</i>			
00-013-0259	Montmorillonite	2	4	2	-
00-043-0685	Illite	19	18	16	-
01-086-1384	Muscovite	-	-	-	12
01-080-0885	Kaolinite	4	3	4	8
01-072-1234	Chlorite	3	3	3	6
01-085-0798	Quartz	36	34	31	44
01-084-1455	Microcline	10	10	4	13
01-076-0927	Albite	7	9	9	11
01-081-2027	Calcite	19	19	30	4
01-083-1766	Dolomite	-	-	1	2

647

648 **Table 3.** Chemical properties of the fine silt and clay (FSi+Cl) fractions isolated from a  
649 coniferous forest soil (CF), a grassland soil (GL), a technosol (TS) and an agricultural  
650 soil (AG).

651

	<b>Organic C</b> (%)	<b>Total N</b> (%)	<b>C/N</b>	<b>Fe</b> (%)
CF FSi+Cl	5.4	0.42	12.9	9.0
GL FSi+Cl	8.5	0.76	11.1	10.3
TS FSi+Cl	0.7	0.08	8.2	7.4
AG FSi+Cl	1.8	0.19	9.5	8.5

652

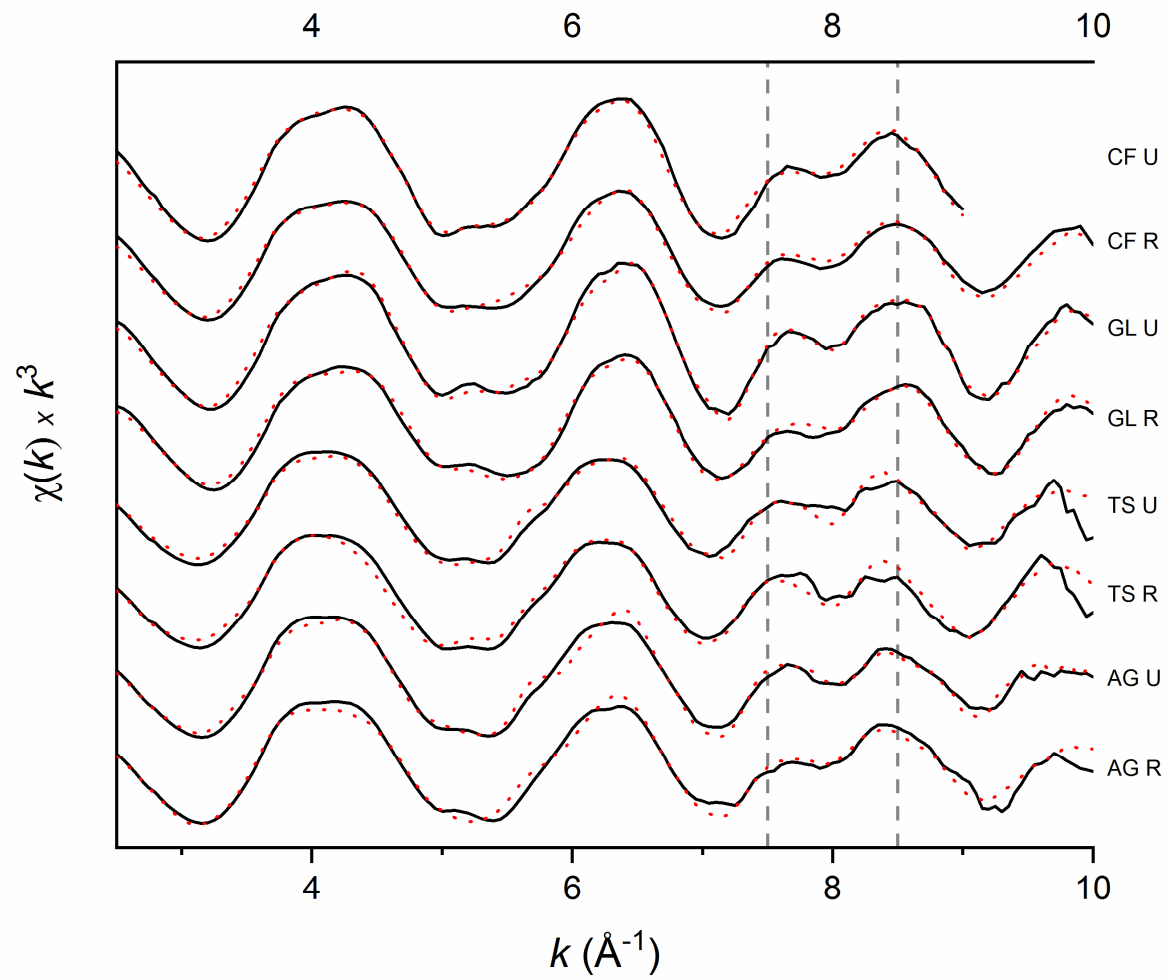
653

**Table 4.** Summary of EXAFS linear combination fitting of fine silt and clay (FSi+Cl) fractions from coniferous forest (CF), grassland (GL), technosol (TS) and agricultural (AG) soils before (U) and after (R) reaction with Fe(III) over the  $k$  range 2.5-10 Å. F-test has been calculated and is reported in column “I”. It denotes the fit improvement using three standards instead of two.

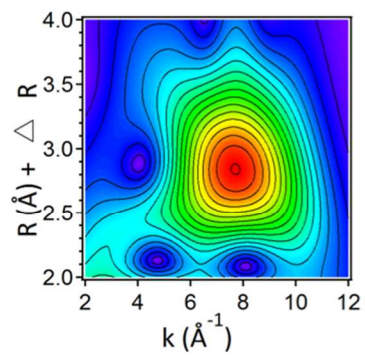
<b>FSi+Cl sample</b>	<b>k range fit</b>	<b>I</b>	<b>Measurements</b>	<b>R-factor</b>	<b>Component 1 Fe-silicates</b>	<b>Component 2 Ferrihydrite</b>	<b>Component 3 Fe(III) citrate</b>	<b>Sum</b>
CF U	2.5-9	0.248	13.414	0.0086	Illite 39%	23%	41%	102%
CF R	2.5-10	0.013	14.369	0.0178	Illite 25%	30%	35%	90%
GL U	2.5-10	0.002	15.324	0.0266	Smectite 31%	48%	18%	97%
GL R	2.5-10	0.001	15.324	0.0387	Smectite 21%	43%	23%	86%
TS U	2.5-10	0.050	15.324	0.0729	Chlorite 26%	47%	33%	105%
TS R	2.5-10	0.025	15.324	0.0959	Chlorite 33%	52%	28%	112%
AG U	2.5-10	0.002	15.324	0.0320	Chlorite 22%	48%	37%	107%
AG R	2.5-10	0.021	15.324	0.0604	Chlorite 14%	41%	46%	102%



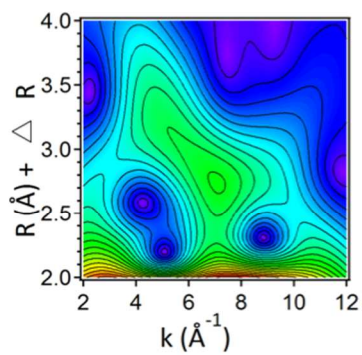
Figure 1.



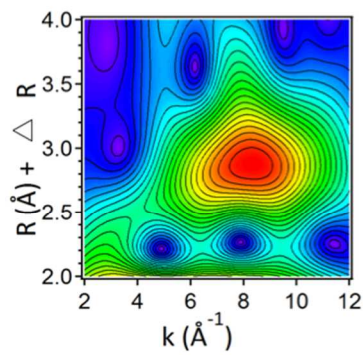
**Figure 2.**



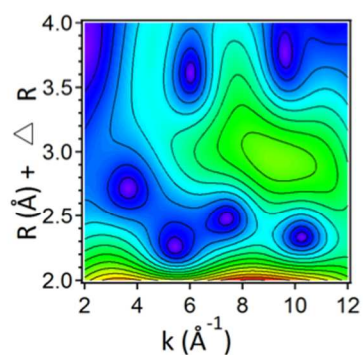
**Ferrihydrite  $\eta = 8, \sigma = 1$**



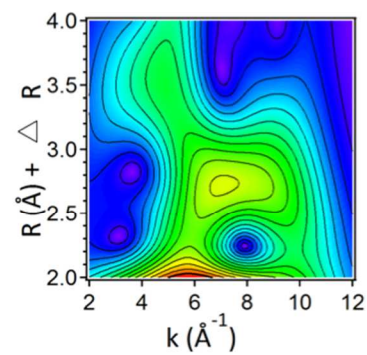
**Fe (III)-citrate  $\eta = 8, \sigma = 1$**



**Illite  $\eta = 8, \sigma = 1$**

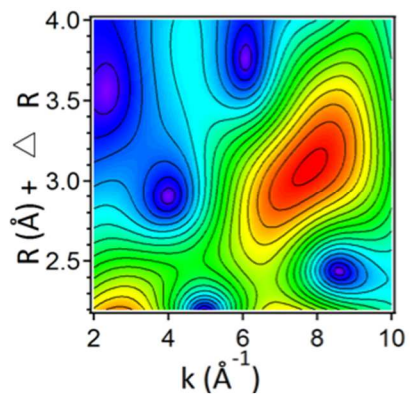


**Smectite  $\eta = 8, \sigma = 1$**

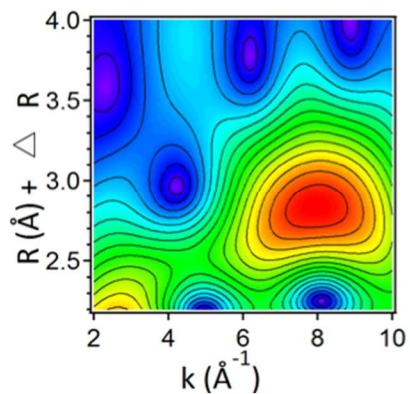


**Chlorite  $\eta = 8, \sigma = 1$**

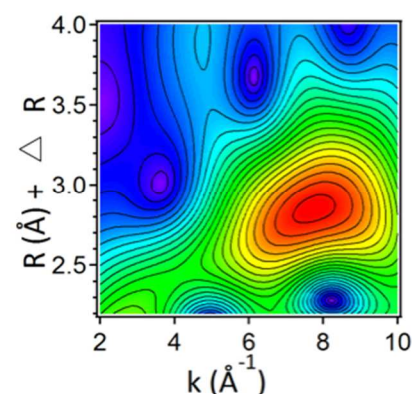
Figure 3.



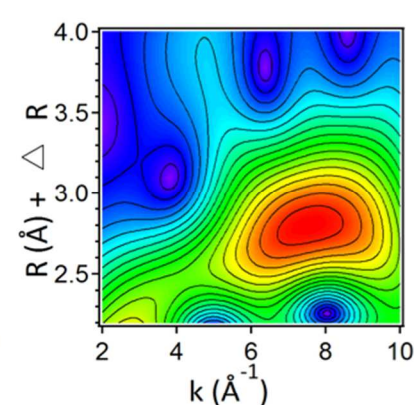
CF U  $\eta = 8, \sigma = 1$



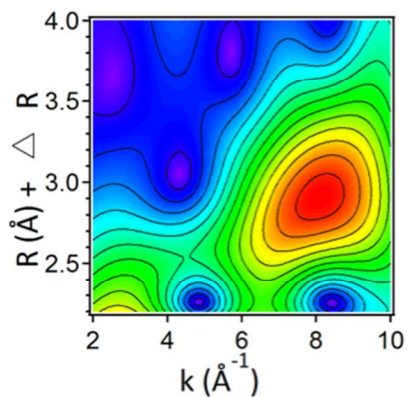
CF R  $\eta = 8, \sigma = 1$



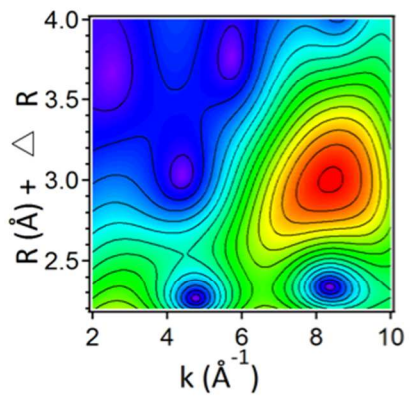
GL U  $\eta = 8, \sigma = 1$



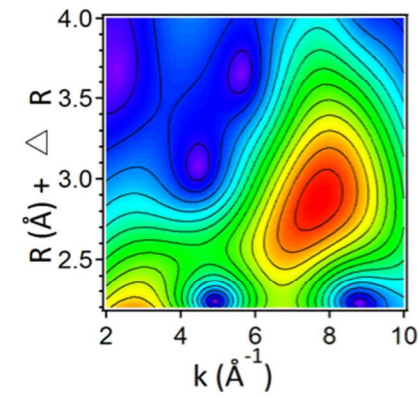
GL R  $\eta = 8, \sigma = 1$



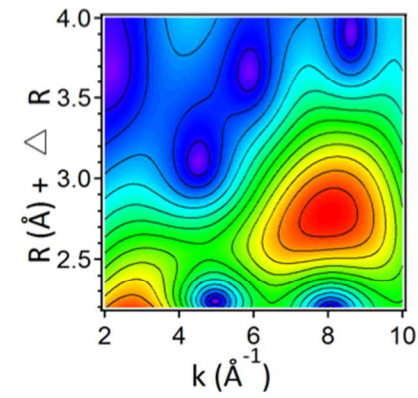
TS U  $\eta = 8, \sigma = 1$



TS R  $\eta = 8, \sigma = 1$



AG U  $\eta = 8, \sigma = 1$



AG R  $\eta = 8, \sigma = 1$