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# Iron-Bound Organic Carbon in Forest Soils: Quantification and Characterization

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## ABSTRACT

Iron oxide minerals play an important role in stabilizing organic carbon (OC) and regulating the biogeochemical cycles of OC on the earth surface. To predict the fate of OC, it is essential to completely understand the amount, spatial variability and characteristics of Fe-bound OC in natural soils. In this study, we investigated the concentrations and characteristics of Fe-bound OC in soils collected from 14 forests in the United States, and determined the impact of ecogeographical variables and soil physicochemical properties on the association of OC and Fe minerals. We found that Fe-bound OC contributed up to 57.8% of total OC (TOC) in forest soils. Atomic ratios of OC:Fe ranged from 0.56 to 17.7 with values of 1-10 for most samples, and these ratios indicate an importance of both sorptive and incorporative interactions. The fraction of Fe-bound OC in TOC ($f_{Fe-OC}$) was not related to the concentration of reactive Fe, which suggests that the importance of association with Fe in OC accumulation was not governed by the concentration of reactive Fe. Concentrations of Fe-bound OC and $f_{Fe-OC}$ increased with the latitude and reached peak values at a site with a mean annual temperature of 6.6 °C. Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) and near-edge X-ray absorption fine structure (NEXAFS) analyses revealed that Fe-bound OC was less aliphatic than non-Fe-bound OC. Fe-

28    bound OC also was more enriched in $^{13}$C compared to the non-Fe-bound OC, but C/N ratios did

29    not differ substantially. In summary, $^{13}$C-enriched OC with less aliphatic carbon and more

30    carboxylic carbon was associated with Fe minerals in the soils, with values of $f_{Fe-OC}$ being

31    controlled by both sorptive and incorporative associations between Fe and OC. Overall, this study

32    demonstrates that Fe oxides play an important role in regulating the biogeochemical cycles of C

33    in forest soils, and uncovers the governing factors for the spatial variability and characteristics of

34    Fe-bound OC.

35

## 1 Introduction

36

37    Soil organic carbon (OC) in forests contributes 40% of the global carbon (C) mass, and is

38    a vital component of C biogeochemical cycles (Eswaran et. al., 1999). Global warming can

39    potentially accelerate the decomposition of forest soil OC, contributing to greenhouse gas

40    emissions (Steffen et al., 1998). Alternatively, forest soils can act as strong sinks for OC, if

41    appropriate management is implemented, such as forest harvesting and fire treatment (Eswaran et.

42    al., 1999; Johnson and Curtis, 2001). Understanding the fate and stability of forest OC is important

43    for evaluating and managing the global C cycle under the framework of climate change.

44    Currently, there is a large information gap concerning the stability and residence time of

45    OC, contributing to the problem that the residence time of OC (ranging from months to hundreds

46    of years) is a major source of uncertainty in modeling and prediction of C cycles (Schmidt et al.,

47    2011; Riley et al., 2014). Many concepts have been proposed to account for OC stabilization and

48    therefore residence times, including molecular recalcitrance, physical occlusion, and chemical

49    protection (Sollins et al., 1996; Krull et al., 2003; Baldock et al., 2004; Mayer et al., 2004;

50    Zimmerman et al., 2004; Schmidt et al., 2011). In general, the stability of OC is regulated by

51    biogeochemical reactions occurring at the interfaces between OC, minerals, and microorganisms,

52    and further knowledge about the mechanism for OC stabilization is critical for building up process-

53    based models to simulate and predict C cycles.

54    A number of lines of evidence suggest a key importance of iron oxide minerals in the

55    stabilization of OC (Kalbitz et al., 2005; Kaiser and Guggenberger, 2007; Wagai and Mayer, 2007).

56    Iron oxides have a relatively high sorption capacity for OC, with sorption coefficients for OC much

57    higher than that of other metal oxides (Kaiser and Guggenberger, 2007; Chorover and Amistadi,

58    2001). Wagai and Mayer (2007) reported Fe-bound OC concentrations in soils up to 22 mg g$^{-1}$ soil,

59    contributing up to 40% of total OC (TOC) for most forest soils. Similarly, Lalonde et al. (2012)

60    found that Fe-bound OC contributed 22% of TOC in sediments. Studies have shown that Fe

61    minerals protect OC from degradation and inhibit mineralization of OC (Baldock and Skjemstad,

62    2000; Kalbitz et al., 2005). There is, however, no systematic study on the occurrence of Fe-bound

63    OC across different forests and its governing factors.

64        The overall goals of this study were to investigate the spatial variability of Fe-bound OC

65    across forest soils and the controlling factors, and to study the characteristics of Fe-bound OC in

66    respect to physicochemical properties of soils. In this study, we first quantified the concentration

67    of Fe-bound OC across 14 forest soils in the United States and analyzed the spatial distribution

68    and influences of ecogeographical factors. Second, we investigated the impact of soil

69    physicochemical properties on the Fe-OC associations. Third, we studied molecular characteristics

70    of Fe-bound OC versus non-Fe-bound OC, including how Fe-OC association influenced the

71    chemical properties of OC and their stable isotope composition. Hence, this study provided a

72    systematic evaluation for the Fe-bound OC in United States forests, the influences of ecological

73    factors on the occurrence of Fe-bound OC, and the effects of association with Fe on the chemical

74    properties of OC.

75

## 2. Methods & Materials

### 2.1 Chemicals and materials

78        Reagents used for Fe reduction experiments include sodium bicarbonate (NaHCO$_3$: Sigma-

79    Aldrich, St. Louis, MO, USA), trisodium citrate dihydrate (Na$_3$C$_6$H$_5$O$_7$•2H$_2$O: Acros Organics,

80    New Jersey, USA), and sodium dithionite (Na$_2$S$_2$O$_4$: Alfa Aesar, Ward Hill, MA, USA). All

81    chemicals used were analytical grade.

82

### 2.2 Soil sample collection, primary characterization and pretreatment

84        Soil samples were collected from 14 forest sites in the United States (Obrist et al., 2011,

85    2012, 2015). The abbreviations and the basic information for the sites are summarized in Table 1.

86    More detailed information on the sites and sampling protocols can be found in previous

87    publications (Obrist et al., 2011, 2012, 2015). Soil texture was analyzed by an ASTM 152-type

88  hydrometer at the Soil Forage and Water Analysis Laboratory at Oklahoma State University

89  (Obrist et al., 2011). The soil pH was measured by mixing soil particle with deionized (DI) water

90  in a solid/solution ratio of 1:1 (Kalra, 1995). Soil samples used in the experiments in this study

91  were ground to < 500 μm and freeze-dried.

92

93  **Table 1**

94

95  **2.3 Total C (TC), TOC and stable C isotope analyses**

96  TC, TOC and stable C isotopic compositions of soil samples were analyzed using a

97  Eurovector elemental analyzer (Eurovector SPA, Milan, Italy) interfaced to a Micromass IsoPrime

98  stable isotope ratio mass spectrometer (Micromass UK Ltd., Manchester, UK). Acetanilide (71.09 %

99  C by weight) was used as a standard compound to establish a calibration curve between mass of C

100  and the m/z 44 response from the mass spectrometer. In this study, the concentration of TC and

101  TOC were expressed as weight %. Stable C isotope analyses were performed after the method of

102  Werner et al. (1999), with results reported in the usual delta notation in units of ‰ vs. Vienna Pee

103  Dee Belemnite (VPDB). For TOC analysis, soil samples were acidified with 1 M HCl with the

104  solution/solid ratio of 1 mL solution/0.5 g soil and heated at 100˚C for 1 hour. The treatment was

105  repeated three times until there was no further effervescence upon acid addition, after which the

106  samples were dried and analyzed.

107

108  **2.4 Nitrogen (N) analysis**

109  The N concentration of each sample was analyzed using a Eurovector elemental analyzer.

110  Acetanilide (10.36 % N by weight) was used as a standard compound to establish a calibration

111  curve between mass of N and the response of the thermal conductivity detector in the elemental

112  analyzer. Total N and non-Fe-bound N concentrations were measured before and after a Fe

113  reduction release treatment for each sample.

114

115  **2.5 Analysis of Fe-bound OC**

116  The concentration of Fe-bound OC was quantified by an established Fe reduction release

117  method, commonly known as DCB extraction involving sodium dithionite, citrate and bicarbonate

118  (Mehra and Jackson, 1960; Wagai and Mayer, 2007; Lalonde et al., 2012). The DCB extraction is

119   assumed to extract most free Fe oxides (i.e. goethite, hematite, ferrihydrite and others) existing in

120   soils, but should not extract structural Fe in clay minerals (Mehra and Jackson, 1960; Wagai and

121   Mayer et al., 2007; Lalonde et al., 2012). In this study, we followed the specific protocol detailed

122   in Lalonde et al. (2012). An aliquot (0.25 g) of soil was mixed with 15 mL of buffer solution at

123   pH 7 (containing 0.11 M bicarbonate and 0.27 M trisodium citrate), and then heated to 80˚C in a

124   water bath. The reducing agent sodium dithionite was added to the samples with final

125   concentration of 0.1 M, and maintained at 80˚C for 15 min. The samples were then centrifuged at

126   10,000 rpm for 10 min, the supernatant was removed, and the residual particles were rinsed using

127   5 mL of DI water. The rinse/centrifuge process was performed three times. The residual particles

128   were freeze-dried and analyzed for TC and TOC concentrations and $\delta^{13}C$ composition. The mass

129   of residual particles were used to calculate the OC concentration associated with non-Fe minerals.

130        The background release of OC during the heating process was measured following the

131   method in Lalonde et al. (2012), where sodium citrate and dithionite were replaced by sodium

132   chloride with the same ionic strength. An aliquot (0.25 g) of dry soil was mixed with 15 mL of 1.6

133   M NaCl and 0.11 M $NaHCO_3$, and heated to 80˚C. Then 0.22 g of NaCl was added, and the solution

134   was maintained at 80˚C for 15 min. The samples were then centrifuged at 10,000 rpm and rinsed

135   three times, and freeze-dried before analysis. The mass of residual particles was used to calculate

136   the concentration of OC released by heating to 80°C. In preliminary experiments, we found that

137   the solution pH increased rapidly during the heating-extraction process with bicarbonate and

138   sodium chloride only, and the increased pH values facilitated the release of additional OC. Hence,

139   we used a lower initial pH of 6 to compensate for the shift to higher pH during heating. To validate

140   the measurement for the concentration of OC released during heating, we also tested the release of

141   OC using a phosphate buffer (same ionic strength) in lieu of the bicarbonate buffer, which can

142   maintain a pH of 7 during heating. Our results showed that the concentration of OC released was

143   similar for both the bicarbonate and phosphate buffer extraction reactions (Supplementary Material,

144   Fig. S1).

145

146   **2.6 Quantification of reactive Fe**

147        The concentration of reactive Fe in soils was determined by analyzing the Fe released

148   during the DCB reduction process. After the reduction treatment, the supernatant of each sample

149   was filtered using a 0.2 μm syringe filter (cellulose acetate), and analyzed for Fe concentration by

150  inductively coupled plasma - atomic emission spectroscopy (Varian-Vista AX CCD, Palo Alto,

151  CA, USA) at an optical absorption wavelength of 259.9 nm.

152

153  **2.7 Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR)**

154      ATR-FTIR analysis to characterize the molecular composition of OC was performed for

155  original soil samples and residual soils after DCB extraction using a Thermo Scientific Nicolet

156  6700 FTIR (Waltham, MA). Dry soil samples were placed directly on the crystal and forced to

157  contact well with the crystal. Spectra were acquired at the resolution of 4 cm$^{-1}$ based on 100 scans.

158  Data collection and baseline correction were accomplished using OMNIC software version 8.3.103.

159

160  **2.8 Near-edge X-ray absorption fine structure (NEXAFS) analysis**

161      For further characterization of chemical structure of OM, carbon (1s) K-edge NEXAFS

162  analyses were performed for select soil samples, i.e. for soils with the highest and lowest values

163  of the fraction of Fe-bound OC to TOC. The soil particles were suspended in DI water and

164  deposited on an Au-coated silicon wafer attached to a Cu sample holder. Before analysis, samples

165  were dried in a vacuum desiccator. The X-ray-based experiments were performed on the Spherical

166  Grating Monochromator (SGM) beamline at the Canadian Light Source (Saskatoon, Canada)

167  (Regier et al., 2007). The energy scale was calibrated using citric acid (absorption at 288.6 eV).

168  Major technical parameters and set-up for the beamline include: X-ray energy ranges 250-2000

169  eV; 45 mm planer undulator; 1000 µm×100 µm spot size; silicon drift detectors (SDD); a titanium

170  filter before the sample; entrance and exit slit gaps of 249.9 µm and 25 µm (Gillespie et al., 2015).

171  Carbon 1s spectra were acquired by slew scans from 270 to 320 eV at 20 s dwell time and 20 scans

172  per sample on a new spot. For data normalization, I$_0$ was collected by measuring the scatter of the

173  incident beam from a freshly Au-coated Si wafer using SDD.  Before the I$_0$ normalization, the pre-

174  edge baseline was adjusted to near zero to remove the scatter in the sample data (Gillespie et al.,

175  2015).

176  **3. Results and Discussion**

177  **3.1 Concentration of Fe-bound OC**

178      This study covered five major forest types in North America, including Spruce-Fir, Pine,

179  Oak, Chaparral, and Maple-beech-birch forests distributed between 29° and 47° N. For the 14

180   forest soils, TC concentrations ranged between 1.5±0.1 and 8.3±2.1% (all percentages given are

181   weight-based), and TOC concentrations ranged between 1.3±0.3 and 6.2±2.9%, which are

182   comparable to values previously reported for North American forests (Wagai and Mayer, 2007;

183   Wilson et al., 2013). Bicarbonate extraction-calibrated Fe-bound OC concentrations ranged from

184   0.3 to 1.9%, with the fraction of Fe-bound OC to TOC ($f_{\text{Fe-OC}}$) averaging 37.8±20.0% (Fig. 1,

185   Supplementary Material, Table S1). Forest HL (Maine) had the highest $f_{\text{Fe-OC}}$ of 57.8%, while

186   forests GS (Florida) and OR (Tennessee) had $f_{\text{Fe-OC}}$ values below detection limits (i.e., below 0.6%).

187   Based on an estimate that 1502 Pg (Pg=1×$10^{15}$ g) of OC is stored in terrestrial soils (Scharlemann,

188   et al., 2014), scaling up these results to a global estimate would yield 538.5±271.5 Pg of Fe-bound

189   OC residing in terrestrial soils. This is a very large pool, e.g. compared to an estimated 19-45 Pg

190   Fe-bound OC that reside in global surface marine sediments (Lalonde et al., 2012). As a major

191   component of the global C pool, Fe-bound OC in terrestrial soils therefore is expected to play a

192   key role in global C biogeochemical cycles.

193

194   **Fig. 1**

195

196   **3.2 Fe-OC association**

197       The values of $f_{\text{Fe-OC}}$ were influenced not only by the concentration of reactive Fe, but also

198   by the type of association between Fe and OC. In this study, the concentration of reactive Fe in

199   forest soils ranged from 0.1 mg g$^{-1}$ to 19.3 mg g$^{-1}$, which is relatively low compared to values of

200   reactive Fe of up to 180 mg g$^{-1}$ reported previously (Wagai and Mayer, 2007; Wagai et al., 2013)

201   (Fig. 2A). A Mollisol in forest sites MS (California) had the highest concentration of reactive Fe,

202   when a Spodosol in forest site GS (Florida) had the lowest reactive Fe concentration. To our

203   surprise, there was no significant correlation between $f_{\text{Fe-OC}}$ and the concentration of reactive Fe

204   (Pearson Correlation Coefficient $r$=−0.418, $p$=0.137, Fig. 2B). This suggests that the proportion of

205   Fe-bound OC is not strongly controlled by the reactive Fe concentration.

206       The OC:Fe molar ratio ranged from 0.56 to 17.7 for all 14 soils, with a value between 1

207   and 10 for 10 soils (Fig. 2A). Previous studies have suggested that the OC:Fe molar ratio can be

208   used as an indicator for the type of association between Fe oxides and OC, with lower values

209   indicating sorptive interactions, while higher values indicate incorporation of OC within Fe oxides

210   (Wagai et al., 2007; Guggenberger and Kaiser, 2003). The highest sorption capacity measured for

211    OC onto Fe oxide corresponds to an OC:Fe molar ratio = 1.0 (Kaiser and Guggenberger, 2006),

212    but by incorporation and co-precipitation of Fe oxide OC:Fe molar ratio can reach much higher

213    values (Guggenberger and Kaiser, 2003). With OC:Fe molar ratios generally between 1-10 for

214    most of the forest soils in this study, we propose that incorporation of OC into Fe oxides plays a

215    major role in the accumulation of Fe-bound OC exceeding sorption by at least a factor of 1 to 10

216    (Wagai and Mayer, 2007; Lalonde, 2012). However, for the HT (Michigan), HL (Maine) and TKF

217    (California) forest soils, the OC:Fe molar ratios were even higher than 10 with maximum value of

218    17.8 (Fig. 2A), implying that incorporation of OC into Fe oxides dominated at these sites. The

219    value of OC:Fe ratio was not related to the concentration of reactive Fe, and varied a lot for soils

220    with similar concentration of total reactive Fe (Fig. 2B). This indicates the type of interactions

221    between OC and Fe was not governed by the amount of Fe. The OC:Fe ratio is potentially regulated

222    by the mineral phases of Fe, as poorly-crystalline Fe oxide has a higher capacity to bind with OC

223    than crystalline Fe minerals (Eusterhues et al., 2014). When sorption dominates the interactions

224    between OC and Fe, OC:Fe can also be influenced greatly by the particle size and surface area of

225    Fe oxides (Gu et al., 1995). Further investigations are needed to determine the factors that control

226    the OC:Fe ratio, and also $f_{\text{Fe-OC}}$ values for soils. Nevertheless, the lack of (or poor) relationship

227    shown here between the concentration of Fe-bound OC and Fe concentrations demonstrates the

228    limitations associated with predicting and modeling the behavior of C in soils based on the Fe

229    concentrations in soils alone.

230

231    **Fig. 2.**

232

233    **3.3 Spatial variance and ecogeographical factors**

234    We analyzed the influences of ecogeographical factors on the occurrence of Fe-bound OC in forest

235    soils (Fig. 3). There was a significant correlation between the TOC concentration and latitude

236    (Pearson correlation coefficient $p$=0.619, $r$=0.018), a pattern commonly observed due to  lower

237    microbial activity and turnover rates of C at higher, colder latitudes (Davidson and Janssens, 2006).

238    Concentration of reactive Fe, if excluding soil MS in California, it was also significantly related

239    to latitude ($p$=0.824, $r$=0.001). Both concentrations of Fe-bound OC and $f_{\text{Fe-OC}}$ also were correlated

240    with latitude ($p$=0.523, $r$=0.053; $p$=0.525, $r$=0.054). Among our samples, soil in forest HL in

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241    Maine, one of the three northern-most site with latitude of 45˚, had the highest $f_{Fe-OC}$ of 57.8%. In

242    forest GS in Florida with lowest latitude of 29.7˚, the $f_{Fe-OC}$ were below detection limits, possibly

243    due to the low concentration of reactive Fe (0.08 mg g$^{-1}$). Hence, increase in latitude both increased

244    concentrations of TOC in soil as well concentrations of Fe-bound OC, suggesting increased

245    interactions between Fe oxide and OC at higher latitudes. There were no clear trends in TOC or

246    Fe-OC interactions with longitude. For elevation, we separated two groups of samples, with one

247    group located below 1000 m (asl) and the other group above (mainly around 2000 and 4000 asl).

248    Concentrations of TOC and Fe-bound OC, however, were not significantly different between the

249    two groups. There were no clear trends with precipitation either, although others have reported

250    positive relationships between mean annual precipitation and soil TOC concentration at a global

251    scale (Amundson, 2001). Both concentrations of Fe-bound OC and $f_{Fe-OC}$ reached highest value

252    with mean annual temperatures at 6.6˚C. The increased annual mean temperature increased the

253    concentration of Fe-bound OC and $f_{Fe-OC}$ when it was below 6.6˚C, then decreased when annual

254    mean temperature was over 6.6˚C. Our results therefore suggest a possibly ideal temperature range

255    where Fe-OC interactions may be particularly pronounced. Finally, the study covered 7 major soil

256    orders, i.e. Alfisols (sample number n=3), Spodosols (n=4), Mollisols (n=1), Inceptisols (n=2),

257    Entisols (n=2), Gelisols (n=1), and Ultisols (n=1). Although there are limited replications in many

258    of these soil orders, highest concentration of Fe-bound OC were observed in Spodosols. Regarding

259    $f_{Fe-OC}$, the ratios also were highest in Spodosols, possibly indicating a particular importance of Fe-

260    bound OC in this soil type which occupies 3.5% of US land areas and 4% global ice-free land (Soil

261    Survey Staff, 1999). However, due to the limited number of samples for each soil order, these

262    findings warrant further confirmation.

263

264    **Fig. 3**

265

266    **3.4 Impact of soil physicochemical properties on Fe-OC association**

267

268            Soil texture can potentially influence the accumulation of Fe-bound OC. Figure 4

269    demonstrates that the fraction of non-calibrated Fe-bound OC showed a significant positive

270    correlation vs. fraction of sand ($r$=0.72, $p$<0.001), and negative correlations vs. fraction of silt ($r$=–

271    0.697, $p$<0.001) and fraction of clay ($r$=–0.616, $p$<0.001). There were similar correlations between

272    labile OC, and the fraction of sand ($r$=0.72, $p$<0.001), silt ($r$=0.72, $p$<0.001) and clay ($r$=0.72,

273    $p$<0.001). However, the calibrated Fe-bound OC had no significant correlation vs. any of the

274    texture fractions. These correlations indicate that the labile OC was mainly associated with the

275    sand component of forest soils, but that the soil texture did not affect the Fe-bound OC. There has

276    been debate on the relative roles of sand, clay and silt in the stabilization of OC in soil (Percival et

277    al., 2000; Six et al., 2002; Eusterhues et al., 2005; Vogel et al., 2014). Eusterhues et al. (2005)

278    found a relationship between the resistance of organic matter to oxidative degradation and the clay

279    concentration in soils, suggesting the importance of clay minerals in the stabilization and

280    accumulation of soil OC. In contrast, Percival et al. (2000) found that the clay mineral fraction

281    explained little of the variation in the accumulation of OC across a range of soil types in New

282    Zealand. Vogel et al. (2014) found that less than 20% of clay mineral surfaces were covered by

283    the sorption of OC, indicating that a limited proportion of clay mineral surface contributed towards

284    the stabilization of OC. Our results suggest that the Fe oxide-mediated stabilization of OC was not

285    related to the size/aggregation-based process, although the labile carbon concentrations increased

286    with the fraction of sand in the soils.

287

288    **Fig. 4**

289

290        The Fe-OC association can also be influenced by the soil pH, which affects the mineral

291    phases of Fe oxides and their surface charge, and their interactions with OC. For our soil samples,

292    the soil pH ranged from 4.1 to 6.3, similar to measurements by Wagai and Mayer (2007) for North

293    America soils. There was no significant correlation between the $f_{Fe-OC}$ and soil pH, e.g. the HL

294    (Maine) soil with pH of 4.4 had the highest $f_{Fe-OC}$ of 57.8%, while the TS(II) (Washington) soil

295    with a similar pH of 4.5 only had a $f_{Fe-OC}$ of 7.4%. For soils with pH ranging from 4.9 to 5.8, $f_{Fe-OC}$

296    did not change correspondingly. Contrastingly, values of OC:Fe molar ratios were significantly

297    influenced by the soil pH; expect for one outlier sample of TS(II) (Washington) soil, there was a

298    significant negative correlation between the OC:Fe molar ratio and soil pH ($r$=-0.477, $p$=0.09)

299    (Supplementary Material, Fig. S2). This may be due to the lower pH values favoring the

300    complexation and precipitation of Fe with OC, while higher pH favors sorptive interactions

301    between Fe minerals and OC (Tipping et al., 2002). If comparing samples with similar pH, the

302    soils with higher TOC had higher OC:Fe molar ratios, e.g. the GS soil (TOC = 1.1%) with pH of

303    4.7 had an OC:Fe molar ratio = 8.5, while the HT (Michigan) soil (TOC = 3.0%) with similar pH

304    of 4.7 had an OC:Fe molar ratio = 17.1. This was consistent with the concept model that the form

305    of Fe was dominated by the Fe-OC co-precipitated complex with relatively higher OC supply

306    (Schwertmann et al., 1986).

307

308    **3.5 Molecular characteristics of Fe-bound OC**

309         The chemical composition of Fe-bound OC can be substantially different from non-Fe-

310    bound OC (Adhikari and Yang, 2015) with broad implications on the C biogeochemical cycles,

311    although such differences so far have received limited attention. We analyzed the difference in

312    chemical composition of Fe-bound OC compared to non-Fe-bound OC using ATR-FTIR analysis

313    (Fig. 5). Overall, there were limited fingerprint peaks for OC, because of the low concentration of

314    TOC and technical challenge for analyzing whole soil particles with FTIR (Calderon et al., 2011;

315    Simonetti et al., 2012). Reeves (2012) demonstrated that FTIR analysis of mineral soils in the

316    ranges of 1600-1750 and 2800-3000 $cm^{-1}$ only can be used to study OC. Peaks in the range of 500-

317    1200 $cm^{-1}$ indicate the presence of clay or other Fe/Al minerals (Fig. 5) (Madejova, 2003; Harsh

318    et al., 2002; Parikh et al., 2014), such as kaolinite or montmorillonite at 850-1200 $cm^{-1}$ (Madejova,

319    2003). Absorption at 850-1200 $cm^{-1}$ can also be due to the presence of polysaccharides, but

320    definitive identification of polysaccharides is not possible in the presence of minerals (Senesi et

321    al., 2003; Tandy et al., 2010). The spectra in the range of 1600-1750 $cm^{-1}$ normally contain

322    fingerprint peaks for functional groups of amides, carboxylates and aromatics (Parikh et al., 2014),

323    but we did not detect any significant peaks in this range. In the range of 2800-3000 $cm^{-1}$, there

324    were no significant peaks for the original soil samples, but after Fe extraction we detected

325    significant peaks at 2850 and 2930 $cm^{-1}$, which are characteristic for the presence of aliphatic

326    carbon. The substantial differences in spectra before and after Fe extraction indicate that aliphatic

327    OC was enriched in the residual soils after extraction. Other functional groups, such as aromatic

328    carbon and hydrophilic functional groups, were more strongly associated with Fe minerals and

329    removed during the Fe extraction, as hydrophilic functional groups can form inner-sphere

330    coordination complexation with iron oxides, and aromatic carbon has electron donor-acceptor

331    interactions with iron oxides (Gu et al., 1995; Axe and Persson, 2001).

332

333    **Fig. 5**

334

335     Furthermore, we analyzed the C 1s NEXAFS spectra of two original, non-extracted soils

336     with the highest and lowest values of $f_{\text{Fe-OC}}$, i.e. HL (Maine) ($f_{\text{Fe-OC}}$=57.8%) and OR (Tennessee)

337     ($f_{\text{Fe-OC}}$ non-detectable) (Supplementary Material, Fig. S3). Three major fingerprint peaks were

338     detected for both soils, including peaks at 285.3, 287.0 and 288.7 eV, which are corresponding to

339     aromatic carbon, aliphatic carbon and carboxylic carbon, respectively (Schumacher et al., 2005;

340     Solomon et al., 2005; Lehmann et al., 2008). The OR (Tennessee) soil had a more substantial

341     signal at 287.0 eV than the HL (Maine) soil, indicating a higher aliphatic carbon concentration in

342     the OR (Tennessee) soil compared to the HL (Maine) soil. Ratio of carboxylic carbon to aromatic

343     carbon (peak height) was 3.8 for HL (Maine) and 1.0 for OR (Tennessee), suggesting that the HL

344     (Maine) soil with higher $f_{\text{Fe-OC}}$ has relatively more carboxylic carbon compared to aromatic carbon.

345     Hence, the C1s NEXAFS spectra suggest that the soil with the higher $f_{\text{Fe-OC}}$ has higher

346     concentration of carboxylic C, while the soil with the lower $f_{\text{Fe-OC value}}$ has a higher aliphatic C

347     concentration. This result is consistent with the comparison of ATR-FTIR spectra in soils before

348     and after Fe extraction, providing evidence that Fe oxides are mainly associated with more

349     hydrophilic and carboxylic carbon, while non-Fe-bound OC was more aliphatic.

350

351     To further investigate the relationships between soil OC and Fe minerals, we analyzed the

352     stable C isotopic compositions ($\delta^{13}C$) of Fe-bound vs. non-Fe-bound OC (i.e., the residual OC

353     after DCB extraction). The $\delta^{13}C$ for original soil samples ranged from –24.5‰ to –27.5‰, and the

354     values for non-Fe-bound OC were –25.1‰ to –28.0‰. The $\delta^{13}C$ for Fe-bound OC was calculated

355     by combined isotope-mass balance (equation (1))

356     $\delta^{13}C_{\text{TOC}} \times TOC = \delta^{13}C_{\text{labile}} \times OC_{\text{labile}} + \delta^{13}C'_{\text{Fe-OC}} \times OC'_{\text{Fe}} + \delta^{13}C_{\text{non-Fe-OC}} \times OC_{\text{non-Fe}}$     (1)

357     where TOC is the concentration of total organic carbon, $OC_{\text{labile}}$ is the concentration of labile OC

358     (extractable by bicarbonate buffer), $OC_{\text{non-Fe}}$ is the concentration of non-Fe-bound OC (residual

359     OC after Fe extraction), and $OC'_{\text{Fe}}$ is the concentration of Fe-bound OC (excluded the labile OC);

360     $\delta^{13}C_{\text{TOC}}$ is $\delta^{13}C$ for bulk OC, $\delta^{13}C_{\text{labile}}$ is $\delta^{13}C$ for labile OC, $\delta^{13}C'_{\text{Fe-OC}}$ is $\delta^{13}C$ for Fe-bound OC,

361     $\delta^{13}C_{\text{non-Fe-OC}}$ is $\delta^{13}C$ for non-Fe-bound OC. However, it is difficult to directly resolve the $\delta^{13}C_{\text{labile}}$

362     and $\delta^{13}C'_{\text{Fe-OC}}$ using this equation. We simplified it to equation (2):

363     $\delta^{13}C_{\text{Fe-OC}} = \dfrac{(\delta^{13}C_{\text{TOC}} \times TOC - \delta^{13}C_{\text{non-Fe-OC}} \times OC_{\text{non-Fe}})}{OC_{\text{Fe}}}$     (2)

364 where $\delta^{13}C_{Fe-OC}$ is $\delta^{13}C$ for Fe-bound OC (including the labile OC), $\delta^{13}C_{TOC}$ is $\delta^{13}C$ for bulk OC,

365 $\delta^{13}C_{non-Fe-OC}$ is $\delta^{13}C$ for non-Fe-bound OC, TOC is the concentration of total organic carbon, $OC_{non-}$

366 $_{Fe}$ is the concentration of non-Fe-bound OC, and $OC_{Fe}$ is the concentration of Fe-bound OC. The

367 $\delta^{13}C$ for Fe-bound OC was heaviest for the TKF (California) soil with a value of –23.0‰, and the

368 lightest for the GS (Florida) forest at –27.0‰. Across all study sites, Fe-bound OC was relatively

369 enriched in $^{13}C$ (1.5±1.2‰ heavier) compared to the non-Fe-bound OC.  However, there is also a

370 contribution of labile OC to the Fe-bound OC, where labile OC is the OC extracted during the

371 dithionite-absent extraction described earlier). The $\delta^{13}C$ value for labile OC can be calculated using

372 equation (3):

373 $$\delta^{13}C_{labile} = \frac{(\delta^{13}C_{TOC} \times TOC - \delta^{13}C_{non-labile} \times OC_{non-labile})}{OC_{labile}} \qquad (3)$$

374 where $\delta^{13}C_{labile}$ is $\delta^{13}C$ for labile OC, $\delta^{13}C_{TOC}$ is $\delta^{13}C$ for bulk OC, $\delta^{13}C_{non-labile}$ is $\delta^{13}C$ for non-labile

375 OC, $OC_{non-labile}$ is the concentration of non-labile OC, and $OC_{labile}$ is the concentration of labile OC.

376 Calculated values of $\delta^{13}C_{labile}$ range from -23.4% to -30.3%, and were lighter than the values for

377 $\delta^{13}C_{Fe-OC}$. Although it is not reliable to quantitatively calculate the $\delta^{13}C$ for Fe-bound OC

378 subtracting the influences of labile OC, these results indicate that the true value for $\delta^{13}C_{Fe-OC}$ should

379 be even somewhat heavier than the results presented in Fig. 6.

380  Our results demonstrate that Fe-bound OC was enriched in $^{13}C$ compared to the non-Fe-

381 bound OC in forest soils, which is consistent with results for sediments, where Fe-bound OC was

382 1.7±2.8‰ heavier than non-Fe-bound OC (Lalonde et al., 2012) (Fig. 6A).  Wang et al. (1998)

383 have shown that $^{13}C$-enriched organic matter in sediments was enriched with O and N (due to the

384 presence of compounds such as proteins and carbohydrate groups), while the lipid fraction was

385 relatively $^{13}C$-depleted. Similarly, compound-specific isotopic analyses have shown that O/N-rich

386 constituents, such as cellulose, hemi-cellulose and amino acids, are $^{13}C$-enriched compared to

387 hydrocarbons (Glaser, 2005), and these $^{13}C$-enriched O/N-rich compounds can associate with Fe

388 oxide extensively through inner-sphere coordination interactions (Parikh et al., 2014). The value

389 of $\Delta^{13}_{FeOC-nonFeOC}$ (= $\delta^{13}C_{Fe-OC}$ - $\delta^{13}C_{non-Fe-OC}$) (difference in $\delta^{13}C$ for Fe-bound OC and non-Fe-

390 bound OC) was inversely correlated with the molar ratio of OC:Fe ($r=-0.53$, $p=0.05$, Fig. 6B).

391 These relationships suggest that the enrichment in $^{13}C$ was to some degree related to the OC:Fe

392 ratio, with lower OC:Fe leading to higher enrichment in $^{13}C$. As discussed previously, lower OC:Fe

393 ratios indicate an increased contribution from sorptive interactions of OC with Fe minerals as

394 compared to incorporation of OC within iron oxides and OC, and these sorptive interactions

395    between O/N-rich organic compounds and Fe oxide results in the enrichment of $^{13}$C of Fe-bound

396    OC vs. non-Fe-bound OC. Previous studies have attributed the stability of relatively labile and

397    reactive compounds, such as amino acids and sugars, to their interactions with minerals (Schmidt

398    et al., 2011), and our results demonstrated the importance of sorption to Fe minerals in increasing

399    the stability of relatively reactive labile compounds.

400

401    **Fig. 6**

402

403        Nitrogen (N)-containing functional groups are potentially important for the association

404    between OC and Fe oxides, although the concentrations of N are much lower than C. The bulk soil

405    contained 0.05-0.45 % N, while the non-Fe-bound component (i.e. the residual solid after DCB

406    extraction) contained 0.06-0.32 % N. Concentrations of Fe-bound N, calculated by difference,

407    ranged up to 0.13 %. However, it is important to note that this number is based without a calibration

408    for labile N that may be removed by the dithionite-free DCB extraction (data not available). There

409    were significant correlations between C and N concentrations for both bulk soils ($r$=0.847, $p$<0.001:

410    Supplementary Material, Fig. S4) and the non-Fe-bound residual components ($r$=0.858, $p$<0.001:

411    Supplementary Material, Fig. S4), with molar C/N ratios of 14.2±2.6 and 13.7±2.3 for bulk and

412    non-Fe-bound OC, respectively. These C/N values are essentially identical to a previously

413    observed molar C/N ratio = 14.3 for a large set of world-wide soils samples (Cleveland et al.,

414    2007), and a molar C/N ratio = 14.4 for OC-rich samples in China (Tian et al., 2010). This result

415    suggests that C/N ratios for Fe-bound OC did not differ from that of non-Fe-bound OC, assuming

416    that the labile carbon did not have a substantially different C/N ratio. Therefore, in contrast to the

417    $^{13}$C enrichment observed for Fe-bound OC, the interactions with Fe minerals did not affect the C/N

418    ratio substantially.

419

420    **4. Conclusion**

421    Fe oxides are recognized as an important mineral phase regulating the amount and characteristics

422    of OM in forest soils. The spatial variability of Fe-bound OC is governed by the geographical

423    factors, such as latitude and annual mean temperature, and also the soil physicochemical properties.

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424 Chemical composition of Fe-bound OC is substantially different from the rest compartment of soil

425 OC. Overall, this study provided a comprehensive investigation into the spatial variance of Fe-

426 bound OC in forest soils, its governing factors, and how the Fe-OC associations affect the chemical

427 characteristics of OC. As a substantial amount of OC was bound to Fe oxides, and association with

428 Fe affected the quality of OC, Fe oxides can regulate the biogeochemical cycles of carbon and its

429 response to climate change.

430

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605 **Figure Captions**

606 **Figure 1.** Concentrations of total carbon (TC), total organic carbon (TOC) and Fe-bound OC in

607 14 forest soils across the United States. Duplicate measurements were conducted for each of two

608 plots in every forest site. Error bars represent standard deviation of measurements of four replicates

609 for each forest site.

610 **Figure 2. A** Concentration of reactive Fe and OC:Fe molar ratio in US forest soils. **B** Relationship

611 between the fraction of Fe-bound OC in TOC ($f_{Fe-OC}$)/ OC:Fe molar ratio and reactive Fe

612 concentration in US forest soils.

613 **Figure 3.** Correlation between the TOC, reactive Fe, concentration of Fe-bound OC, $f_{Fe-TOC}$, OC:Fe

614 and ecogeographical parameters including latitude, longitude, elevation (asl), precipitation (mean

615 annual) and temperature (annual mean).

616 **Figure 4.** Correlation of the fractions of iron-bound organic carbon (uncalibrated and calibrated

617 for loss of labile OC) and labile organic carbon vs. fractions of sand, silt, and clay in forest soils.

618 **Figure 5**. Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR)

619 analysis for representative forest soils before (black line) and after Fe extraction (red line). All the

620 spectra are background-calibrated. Among the 14 forest soils sampled in this study, we used five

621 different forest soils, with $f_{Fe-OC}$ ranging 5.6-57.8%.

622 **Figure 6. A**. $\delta^{13}C$ of total organic carbon and non-iron bound organic carbon for 14 U.S. forest

623 sites. **B**. Correlation between $\Delta^{13}_{FeOC-nonFeOC}$ and molar ratio of OC:Fe.

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**Table 1 Information for the 14 forest sites studied (Obrist et al., 2011, 2012, 2015)**

| Forest ID | Abbr. | Location | Soil Order | Climate Zone | Precip.[a] (mm y$^{-1}$) | Temp[b] (ºC) | LAT(°)[c] | LONG(°)[d] | Elevation (m asl) |
|---|---|---|---|---|---|---|---|---|---|
| Ashland | **AL** | Ashland, Missouri | Alfisols | Humid Continental | 1023 | 13.9 | 38.73 | -92.20 | 210 |
| Bartlett | **BL** | Bartlett, New Hampshire | Spodosols | Humid Continental | 1300 | 4.5 | 44.0 | -71.29 | 272 |
| Marysville | **MS** | Marysville, California | Mollisols | Mediterranean climate | 775 | 16.9 | 39.25 | -121.28 | 386 |
| Gainesville | **GS** | Gainesville, Florida | Spodosols | Humid Subtropical | 1228 | 21.7 | 29.74 | -82.22 | 50 |
| Oak Ridge | **OR** | Oak Ridge, Tennessee | Ultisols | Humid Subtropical | 1350 | 14.5 | 35.97 | -84,28 | |
| Little Valley (post-fire) | **LVF** | Little Valley, Nevada | Entisols | Highland Climate | 551 | 5.0 | 39.12 | -119.93 | 2010 |
| Little Valley | **LV** | Little Valley, Nevada | Entisols | Highland Climate | 550 | 5.0 | 39.12 | -119.93 | 2011 |
| Truckee (post-fire) | **TKF** | Truckee, California | Alfisols | Highland Climate | 569 | 6.0 | 39.37 | -120.1 | 1768 |
| Truckee | **TK** | Truckee, California | Alfisols | Highland Climate | 568 | 5.9 | 39.37 | -120.1 | 1767 |
| Niwot Ridge | **NR** | Niwot Ridge, Colorado | Alfisols | Highland Climate | 800 | 1.3 | 40.03 | -105.55 | 3050 |
| Hart | **HT** | Hart, Michigan | Spodosols | Humid Continental | 812 | 7.6 | 43.67 | -86.15 | 210 |
| Howland | **HL** | Howland, Maine | Spodosols | Humid Continental | 1040 | 6.7 | 45.20 | -68.74 | 60 |
| Thompson I | **TSI** | Ravensdale, Washington | Inceptisols | Highland Climate | 1141 | 9.8 | 47.38 | -121.93 | 221 |
| Thompson II | **TSII** | Ravensdale, Washington | Inceptisols | Highland Climate | 1140 | 9.8 | 47.38 | -121.93 | 220 |

a: annual precipitation; b: annual mean temperature; c latitude; d: longitude.

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**Fig. 1**

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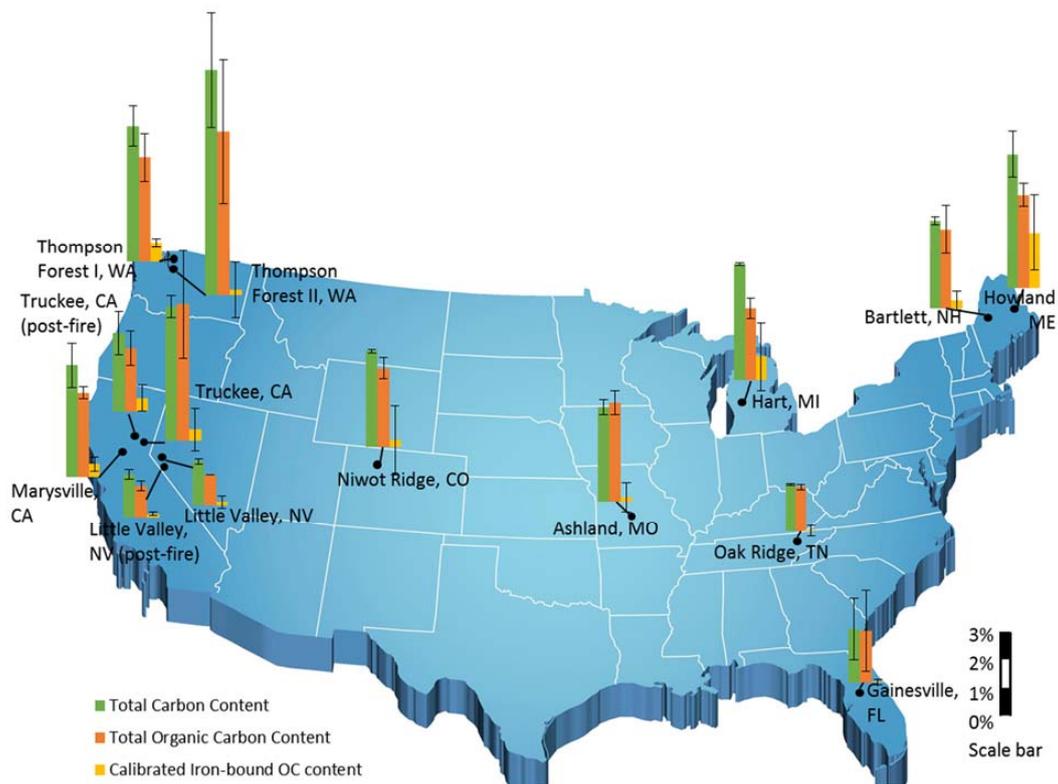
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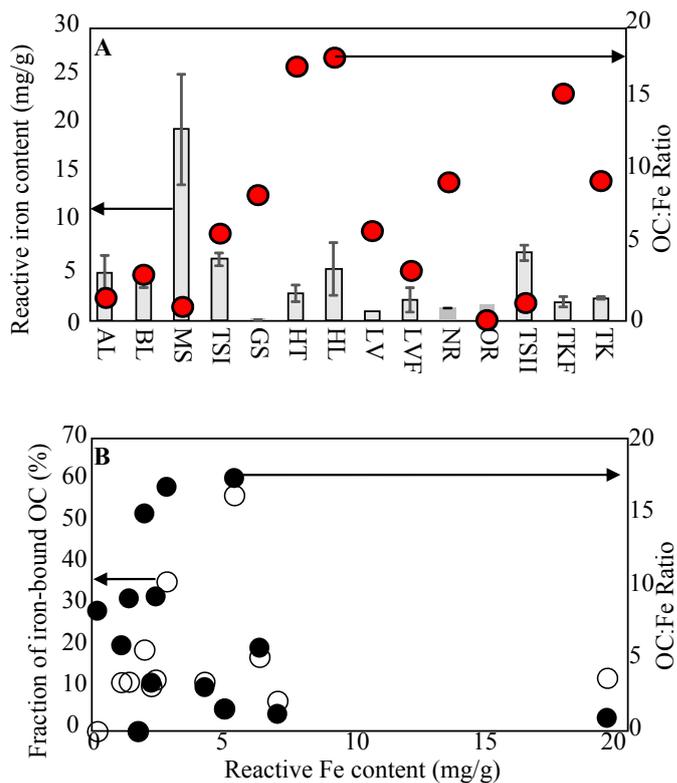
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647 **Fig. 2**

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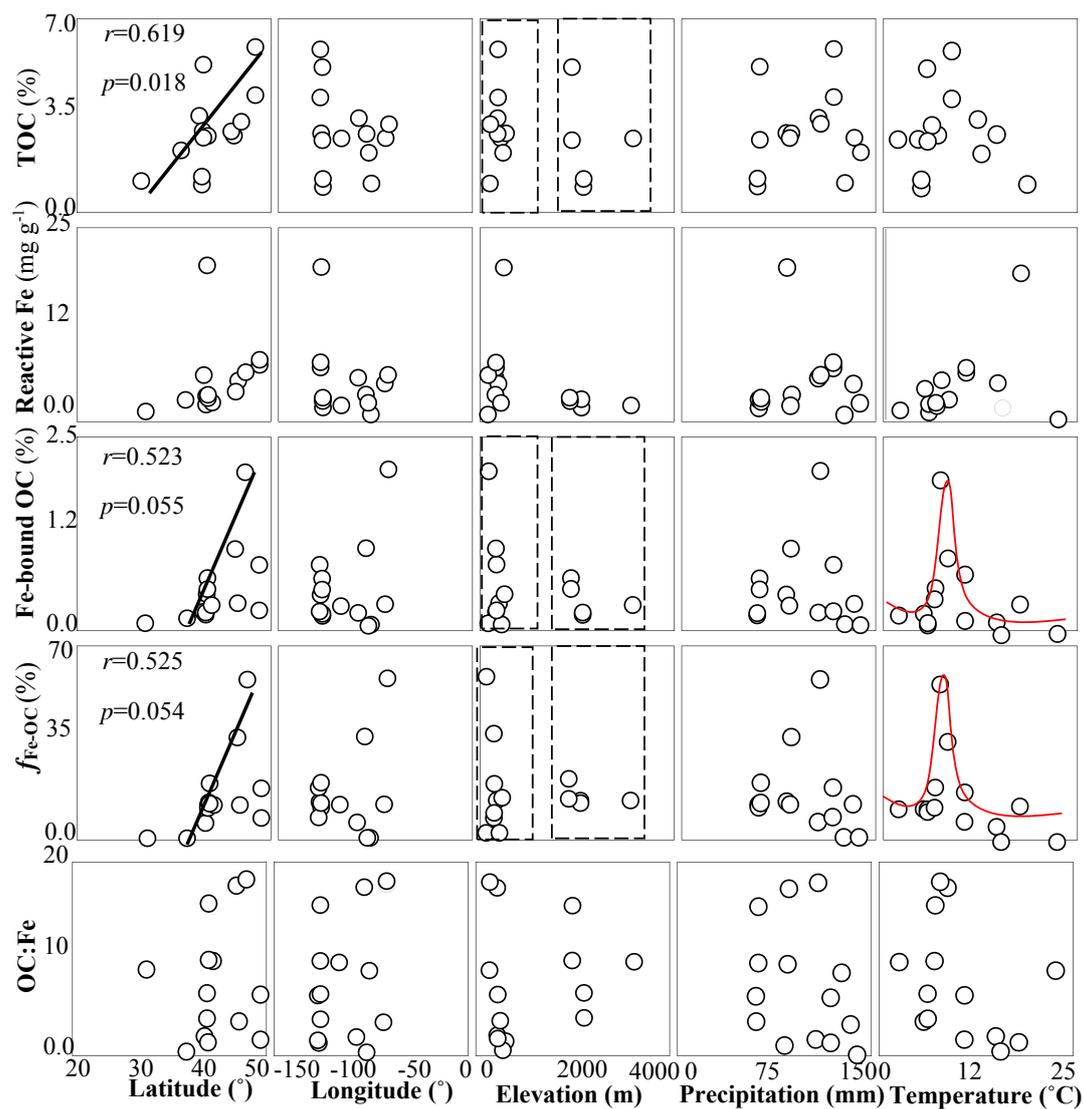
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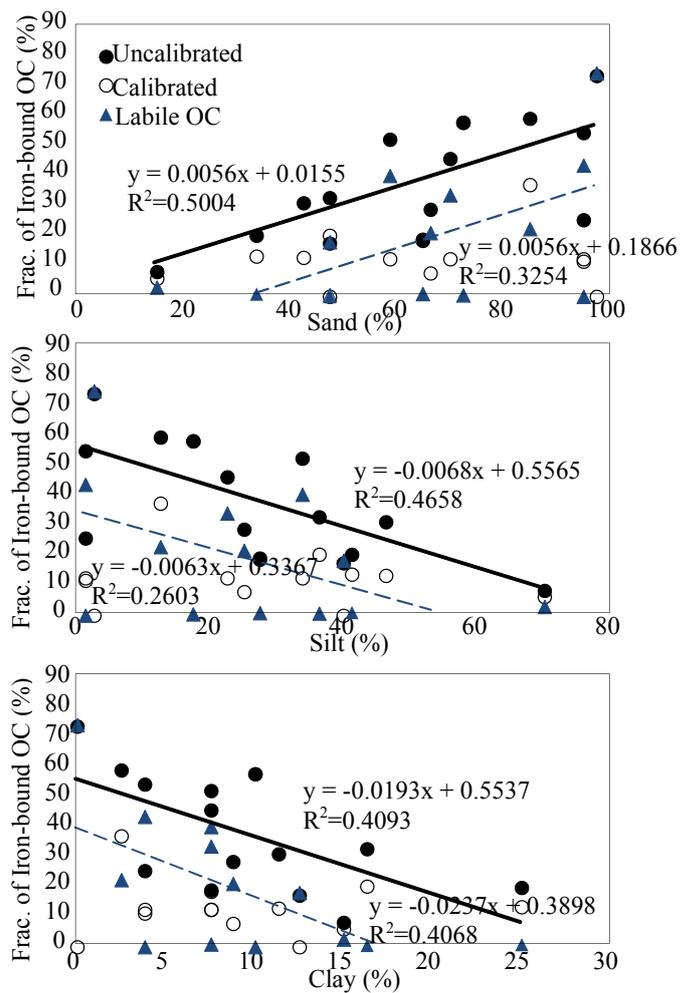
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**Fig. 3**

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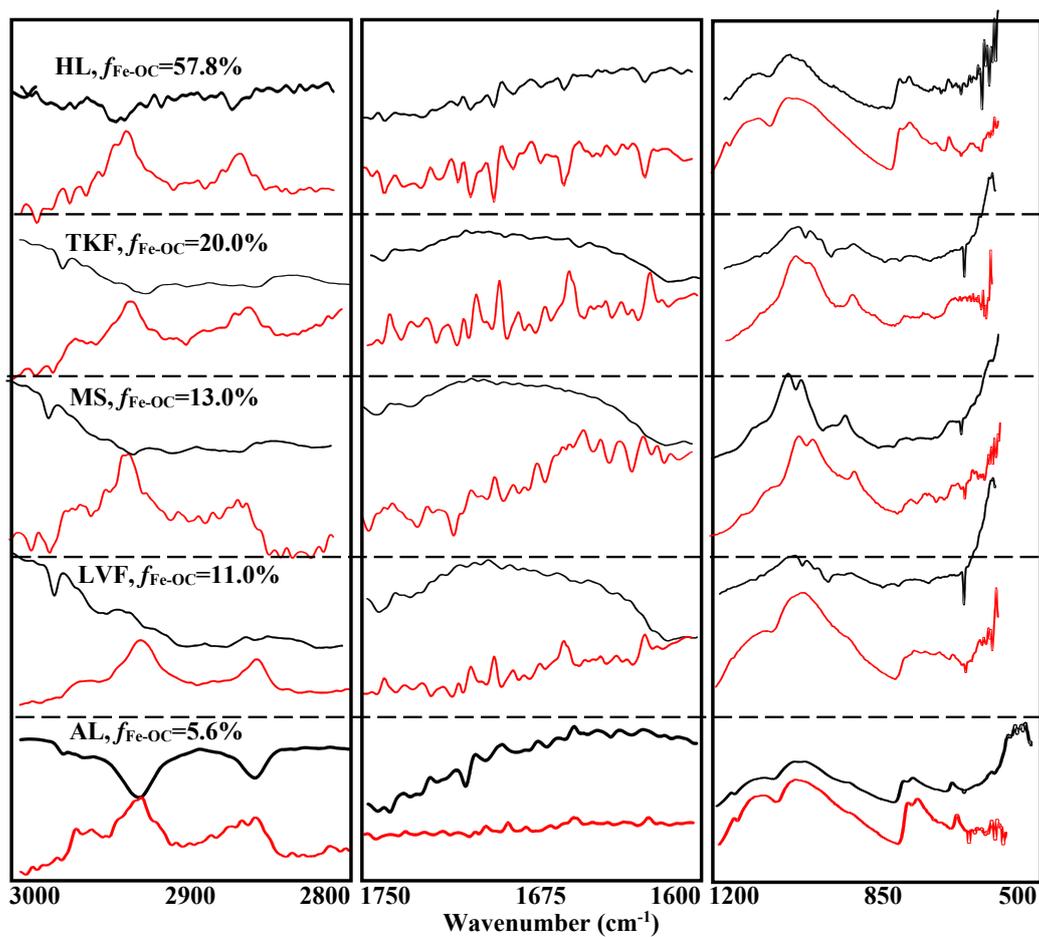
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669     **Fig. 4**

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**Fig. 5**
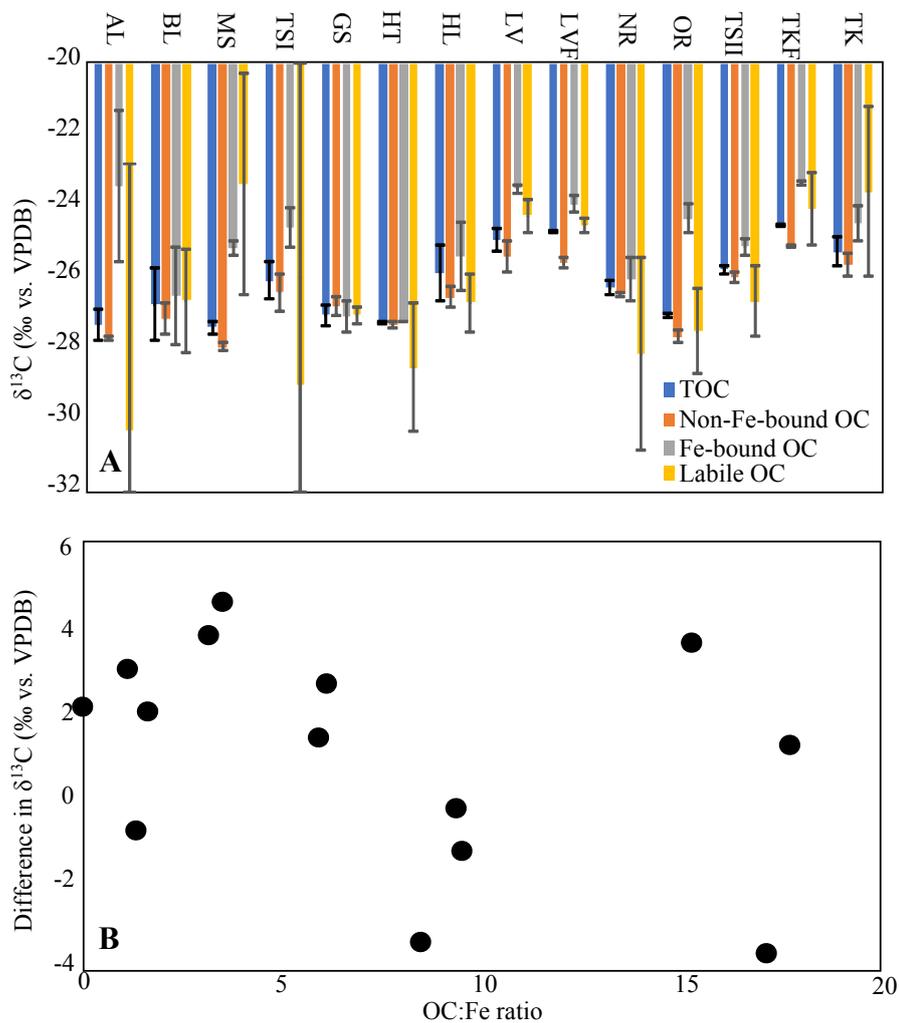
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692 **Fig. 6**

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