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

Qian Zhao¹, Simon R Poulson², Daniel Obrist³, Samira Sumaila⁴,⁵, James J. Dynes⁴, Joyce M. McBeth⁴,⁵, Yu Yang¹*

[1] {Department of Civil and Environmental Engineering, University of Nevada, Reno, Nevada, 89557}
[2] {Department of Geological Sciences and Engineering, University of Nevada, Reno, Nevada, 89557}
[3] {Division of Atmospheric Sciences, Desert Research Institute, Reno, Nevada, 89512}
[4] {Canadian Light Source, 44 Innovation Blvd, Saskatoon, SK, S7N 2V3, Canada}
[5] {Department of Geological Sciences, University of Saskatchewan, Saskatoon, SK, S7N 5E2, Canada}

* Correspondence to: Y. Yang (yuy@unr.edu)

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-
bound OC also was more enriched in $^{13}$C compared to the non-Fe-bound OC, but C/N ratios did not differ substantially. In summary, $^{13}$C-enriched OC with less aliphatic carbon and more carboxylic carbon was associated with Fe minerals in the soils, with values of $f_{\text{Fe-OC}}$ being controlled by both sorptive and incorporative associations between Fe and OC. Overall, this study demonstrates that Fe oxides play an important role in regulating the biogeochemical cycles of C in forest soils, and uncovers the governing factors for the spatial variability and characteristics of Fe-bound OC.

1 Introduction

Soil organic carbon (OC) in forests contributes 40% of the global carbon (C) mass, and is a vital component of C biogeochemical cycles (Eswaran et. al., 1999). Global warming can potentially accelerate the decomposition of forest soil OC, contributing to greenhouse gas emissions (Steffen et al., 1998). Alternatively, forest soils can act as strong sinks for OC, if appropriate management is implemented, such as forest harvesting and fire treatment (Eswaran et. al., 1999; Johnson and Curtis, 2001). Understanding the fate and stability of forest OC is important for evaluating and managing the global C cycle under the framework of climate change.

Currently, there is a large information gap concerning the stability and residence time of OC, contributing to the problem that the residence time of OC (ranging from months to hundreds of years) is a major source of uncertainty in modeling and prediction of C cycles (Schmidt et al., 2011; Riley et al., 2014). Many concepts have been proposed to account for OC stabilization and therefore residence times, including molecular recalcitrance, physical occlusion, and chemical protection (Sollins et al., 1996; Krull et al., 2003; Baldock et al., 2004; Mayer et al., 2004; Zimmerman et al., 2004; Schmidt et al., 2011). In general, the stability of OC is regulated by biogeochemical reactions occurring at the interfaces between OC, minerals, and microorganisms, and further knowledge about the mechanism for OC stabilization is critical for building up process-based models to simulate and predict C cycles.

A number of lines of evidence suggest a key importance of iron oxide minerals in the stabilization of OC (Kalbitz et al., 2005; Kaiser and Guggenberger, 2007; Wagai and Mayer, 2007). Iron oxides have a relatively high sorption capacity for OC, with sorption coefficients for OC much higher than that of other metal oxides (Kaiser and Guggenberger, 2007; Chorover and Amistadi,
2001). Wagai and Mayer (2007) reported Fe-bound OC concentrations in soils up to 22 mg g\(^{-1}\) soil, contributing up to 40% of total OC (TOC) for most forest soils. Similarly, Lalonde et al. (2012) found that Fe-bound OC contributed 22% of TOC in sediments. Studies have shown that Fe minerals protect OC from degradation and inhibit mineralization of OC (Baldock and Skjemstad, 2000; Kalbitz et al., 2005). There is, however, no systematic study on the occurrence of Fe-bound OC across different forests and its governing factors.

The overall goals of this study were to investigate the spatial variability of Fe-bound OC across forest soils and the controlling factors, and to study the characteristics of Fe-bound OC in respect to physicochemical properties of soils. In this study, we first quantified the concentration of Fe-bound OC across 14 forest soils in the United States and analyzed the spatial distribution and influences of ecogeographical factors. Second, we investigated the impact of soil physicochemical properties on the Fe-OC associations. Third, we studied molecular characteristics of Fe-bound OC versus non-Fe-bound OC, including how Fe-OC association influenced the chemical properties of OC and their stable isotope composition. Hence, this study provided a systematic evaluation for the Fe-bound OC in United States forests, the influences of ecological factors on the occurrence of Fe-bound OC, and the effects of association with Fe on the chemical properties of OC.

2. Methods & Materials

2.1 Chemicals and materials

Reagents used for Fe reduction experiments include sodium bicarbonate (NaHCO\(_3\): Sigma-Aldrich, St. Louis, MO, USA), trisodium citrate dihydrate (Na\(_3\)C\(_6\)H\(_5\)O\(_7\)\(\cdot\)2H\(_2\)O: Acros Organics, New Jersey, USA), and sodium dithionite (Na\(_2\)S\(_2\)O\(_4\): Alfa Aesar, Ward Hill, MA, USA). All chemicals used were analytical grade.

2.2 Soil sample collection, primary characterization and pretreatment

Soil samples were collected from 14 forest sites in the United States (Obrist et al., 2011, 2012, 2015). The abbreviations and the basic information for the sites are summarized in Table 1. More detailed information on the sites and sampling protocols can be found in previous publications (Obrist et al., 2011, 2012, 2015). Soil texture was analyzed by an ASTM 152-type
hydrometer at the Soil Forage and Water Analysis Laboratory at Oklahoma State University (Obrist et al., 2011). The soil pH was measured by mixing soil particle with deionized (DI) water in a solid/solution ratio of 1:1 (Kalra, 1995). Soil samples used in the experiments in this study were ground to < 500 µm and freeze-dried.

Table 1

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

TC, TOC and stable C isotopic compositions of soil samples were analyzed using a Eurovector elemental analyzer (Eurovector SPA, Milan, Italy) interfaced to a Micromass IsoPrime stable isotope ratio mass spectrometer (Micromass UK Ltd., Manchester, UK). Acetanilide (71.09 % C by weight) was used as a standard compound to establish a calibration curve between mass of C and the m/z 44 response from the mass spectrometer. In this study, the concentration of TC and TOC were expressed as weight %. Stable C isotope analyses were performed after the method of Werner et al. (1999), with results reported in the usual delta notation in units of ‰ vs. Vienna Pee Dee Belemnite (VPDB). For TOC analysis, soil samples were acidified with 1 M HCl with the solution/solid ratio of 1 mL solution/0.5 g soil and heated at 100°C for 1 hour. The treatment was repeated three times until there was no further effervescence upon acid addition, after which the samples were dried and analyzed.

2.4 Nitrogen (N) analysis

The N concentration of each sample was analyzed using a Eurovector elemental analyzer. Acetanilide (10.36 % N by weight) was used as a standard compound to establish a calibration curve between mass of N and the response of the thermal conductivity detector in the elemental analyzer. Total N and non-Fe-bound N concentrations were measured before and after a Fe reduction release treatment for each sample.

2.5 Analysis of Fe-bound OC

The concentration of Fe-bound OC was quantified by an established Fe reduction release method, commonly known as DCB extraction involving sodium dithionite, citrate and bicarbonate (Mehra and Jackson, 1960; Wagai and Mayer, 2007; Lalonde et al., 2012). The DCB extraction is
assumed to extract most free Fe oxides (i.e. goethite, hematite, ferrihydrite and others) existing in soils, but should not extract structural Fe in clay minerals (Mehra and Jackson, 1960; Wagai and Mayer et al., 2007; Lalonde et al., 2012). In this study, we followed the specific protocol detailed in Lalonde et al. (2012). An aliquot (0.25 g) of soil was mixed with 15 mL of buffer solution at pH 7 (containing 0.11 M bicarbonate and 0.27 M trisodium citrate), and then heated to 80°C in a water bath. The reducing agent sodium dithionite was added to the samples with final concentration of 0.1 M, and maintained at 80°C for 15 min. The samples were then centrifuged at 10,000 rpm for 10 min, the supernatant was removed, and the residual particles were rinsed using 5 mL of DI water. The rinse/centrifuge process was performed three times. The residual particles were freeze-dried and analyzed for TC and TOC concentrations and δ^{13}C composition. The mass of residual particles were used to calculate the OC concentration associated with non-Fe minerals.

The background release of OC during the heating process was measured following the method in Lalonde et al. (2012), where sodium citrate and dithionite were replaced by sodium chloride with the same ionic strength. An aliquot (0.25 g) of dry soil was mixed with 15 mL of 1.6 M NaCl and 0.11 M NaHCO_{3}, and heated to 80°C. Then 0.22 g of NaCl was added, and the solution was maintained at 80°C for 15 min. The samples were then centrifuged at 10,000 rpm and rinsed three times, and freeze-dried before analysis. The mass of residual particles was used to calculate the concentration of OC released by heating to 80°C. In preliminary experiments, we found that the solution pH increased rapidly during the heating-extraction process with bicarbonate and sodium chloride only, and the increased pH values facilitated the release of additional OC. Hence, we used a lower initial pH of 6 to compensate for the shift to higher pH during heating. To validate the measurement for the concentration of OC released during heating, we also tested the release of OC using a phosphate buffer (same ionic strength) in lieu of the bicarbonate buffer, which can maintain a pH of 7 during heating. Our results showed that the concentration of OC released was similar for both the bicarbonate and phosphate buffer extraction reactions (Supplementary Material, Fig. S1).

### 2.6 Quantification of reactive Fe

The concentration of reactive Fe in soils was determined by analyzing the Fe released during the DCB reduction process. After the reduction treatment, the supernatant of each sample was filtered using a 0.2 μm syringe filter (cellulose acetate), and analyzed for Fe concentration by
inductively coupled plasma - atomic emission spectroscopy (Varian-Vista AX CCD, Palo Alto, CA, USA) at an optical absorption wavelength of 259.9 nm.

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

ATR-FTIR analysis to characterize the molecular composition of OC was performed for original soil samples and residual soils after DCB extraction using a Thermo Scientific Nicolet 6700 FTIR (Waltham, MA). Dry soil samples were placed directly on the crystal and forced to contact well with the crystal. Spectra were acquired at the resolution of 4 cm⁻¹ based on 100 scans. Data collection and baseline correction were accomplished using OMNIC software version 8.3.103.

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

For further characterization of chemical structure of OM, carbon (1s) K-edge NEXAFS analyses were performed for select soil samples, i.e. for soils with the highest and lowest values of the fraction of Fe-bound OC to TOC. The soil particles were suspended in DI water and deposited on an Au-coated silicon wafer attached to a Cu sample holder. Before analysis, samples were dried in a vacuum desiccator. The X-ray-based experiments were performed on the Spherical Grating Monochromator (SGM) beamline at the Canadian Light Source (Saskatoon, Canada) (Regier et al., 2007). The energy scale was calibrated using citric acid (absorption at 288.6 eV). Major technical parameters and set-up for the beamline include: X-ray energy ranges 250-2000 eV; 45 mm planer undulator; 1000 μm×100 μm spot size; silicon drift detectors (SDD); a titanium filter before the sample; entrance and exit slit gaps of 249.9 μm and 25 μm (Gillespie et al., 2015). Carbon 1s spectra were acquired by slew scans from 270 to 320 eV at 20 s dwell time and 20 scans per sample on a new spot. For data normalization, I₀ was collected by measuring the scatter of the incident beam from a freshly Au-coated Si wafer using SDD. Before the I₀ normalization, the pre-edge baseline was adjusted to near zero to remove the scatter in the sample data (Gillespie et al., 2015).

3. Results and Discussion

3.1 Concentration of Fe-bound OC

This study covered five major forest types in North America, including Spruce-Fir, Pine, Oak, Chaparral, and Maple-beech-birch forests distributed between 29° and 47° N. For the 14
forest soils, TC concentrations ranged between 1.5±0.1 and 8.3±2.1% (all percentages given are weight-based), and TOC concentrations ranged between 1.3±0.3 and 6.2±2.9%, which are comparable to values previously reported for North American forests (Wagai and Mayer, 2007; Wilson et al., 2013). Bicarbonate extraction-calibrated Fe-bound OC concentrations ranged from 0.3 to 1.9%, with the fraction of Fe-bound OC to TOC ($f_{Fe-OC}$) averaging 37.8±20.0% (Fig. 1, Supplementary Material, Table S1). Forest HL (Maine) had the highest $f_{Fe-OC}$ of 57.8%, while forests GS (Florida) and OR (Tennessee) had $f_{Fe-OC}$ values below detection limits (i.e., below 0.6%).

Based on an estimate that 1502 Pg ($Pg=1\times10^{15}$ g) of OC is stored in terrestrial soils (Scharlemann, et al., 2014), scaling up these results to a global estimate would yield 538.5±271.5 Pg of Fe-bound OC residing in terrestrial soils. This is a very large pool, e.g. compared to an estimated 19-45 Pg Fe-bound OC that reside in global surface marine sediments (Lalonde et al., 2012). As a major component of the global C pool, Fe-bound OC in terrestrial soils therefore is expected to play a key role in global C biogeochemical cycles.

Fig. 1

3.2 Fe-OC association

The values of $f_{Fe-OC}$ were influenced not only by the concentration of reactive Fe, but also by the type of association between Fe and OC. In this study, the concentration of reactive Fe in forest soils ranged from 0.1 mg g$^{-1}$ to 19.3 mg g$^{-1}$, which is relatively low compared to values of reactive Fe of up to 180 mg g$^{-1}$ reported previously (Wagai and Mayer, 2007; Wagai et al., 2013) (Fig. 2A). A Mollisol in forest sites MS (California) had the highest concentration of reactive Fe, when a Spodosol in forest site GS (Florida) had the lowest reactive Fe concentration. To our surprise, there was no significant correlation between $f_{Fe-OC}$ and the concentration of reactive Fe (Pearson Correlation Coefficient $r=-0.418$, $p=0.137$, Fig. 2B). This suggests that the proportion of Fe-bound OC is not strongly controlled by the reactive Fe concentration.

The OC:Fe molar ratio ranged from 0.56 to 17.7 for all 14 soils, with a value between 1 and 10 for 10 soils (Fig. 2A). Previous studies have suggested that the OC:Fe molar ratio can be used as an indicator for the type of association between Fe oxides and OC, with lower values indicating sorptive interactions, while higher values indicate incorporation of OC within Fe oxides (Wagai et al., 2007; Guggenberger and Kaiser, 2003). The highest sorption capacity measured for...
OC onto Fe oxide corresponds to an OC:Fe molar ratio = 1.0 (Kaiser and Guggenberger, 2006), but by incorporation and co-precipitation of Fe oxide OC:Fe molar ratio can reach much higher values (Guggenberger and Kaiser, 2003). With OC:Fe molar ratios generally between 1-10 for most of the forest soils in this study, we propose that incorporation of OC into Fe oxides plays a major role in the accumulation of Fe-bound OC exceeding sorption by at least a factor of 1 to 10 (Wagai and Mayer, 2007; Lalonde, 2012). However, for the HT (Michigan), HL (Maine) and TKF (California) forest soils, the OC:Fe molar ratios were even higher than 10 with maximum value of 17.8 (Fig. 2A), implying that incorporation of OC into Fe oxides dominated at these sites. The value of OC:Fe ratio was not related to the concentration of reactive Fe, and varied a lot for soils with similar concentration of total reactive Fe (Fig. 2B). This indicates the type of interactions between OC and Fe was not governed by the amount of Fe. The OC:Fe ratio is potentially regulated by the mineral phases of Fe, as poorly-crystalline Fe oxide has a higher capacity to bind with OC than crystalline Fe minerals (Eusterhues et al., 2014). When sorption dominates the interactions between OC and Fe, OC:Fe can also be influenced greatly by the particle size and surface area of Fe oxides (Gu et al., 1995). Further investigations are needed to determine the factors that control the OC:Fe ratio, and also \( f_{\text{Fe-OC}} \) values for soils. Nevertheless, the lack of (or poor) relationship shown here between the concentration of Fe-bound OC and Fe concentrations demonstrates the limitations associated with predicting and modeling the behavior of C in soils based on the Fe concentrations in soils alone.

Fig. 2.

3.3 Spatial variance and ecogeographical factors

We analyzed the influences of ecogeographical factors on the occurrence of Fe-bound OC in forest soils (Fig. 3). There was a significant correlation between the TOC concentration and latitude (Pearson correlation coefficient \( p=0.619, r=0.018 \), a pattern commonly observed due to lower microbial activity and turnover rates of C at higher, colder latitudes (Davidson and Janssens, 2006). Concentration of reactive Fe, if excluding soil MS in California, it was also significantly related to latitude (\( p=0.824, r=0.001 \)). Both concentrations of Fe-bound OC and \( f_{\text{Fe-OC}} \) also were correlated with latitude (\( p=0.523, r=0.053; p=0.525, r=0.054 \)). Among our samples, soil in forest HL in
Maine, one of the three northern-most sites with latitude of 45°, had the highest $f_{Fe-OC}$ of 57.8%. In forest GS in Florida with lowest latitude of 29.7°, the $f_{Fe-OC}$ were below detection limits, possibly due to the low concentration of reactive Fe (0.08 mg g$^{-1}$). Hence, increase in latitude both increased concentrations of TOC in soil as well concentrations of Fe-bound OC, suggesting increased interactions between Fe oxide and OC at higher latitudes. There were no clear trends in TOC or Fe-OC interactions with longitude. For elevation, we separated two groups of samples, with one group located below 1000 m (asl) and the other group above (mainly around 2000 and 4000 asl). Concentrations of TOC and Fe-bound OC, however, were not significantly different between the two groups. There were no clear trends with precipitation either, although others have reported positive relationships between mean annual precipitation and soil TOC concentration at a global scale (Amundson, 2001). Both concentrations of Fe-bound OC and $f_{Fe-OC}$ reached highest value with mean annual temperatures at 6.6°C. The increased annual mean temperature increased the concentration of Fe-bound OC and $f_{Fe-OC}$ when it was below 6.6°C, then decreased when annual mean temperature was over 6.6°C. Our results therefore suggest a possibly ideal temperature range where Fe-OC interactions may be particularly pronounced. Finally, the study covered 7 major soil orders, i.e. Alfisols (sample number n=3), Spodosols (n=4), Mollisols (n=1), Inceptisols (n=2), Entisols (n=2), Gelisols (n=1), and Ultisols (n=1). Although there are limited replications in many of these soil orders, highest concentration of Fe-bound OC were observed in Spodosols. Regarding $f_{Fe-OC}$, the ratios also were highest in Spodosols, possibly indicating a particular importance of Fe-bound OC in this soil type which occupies 3.5% of US land areas and 4% global ice-free land (Soil Survey Staff, 1999). However, due to the limited number of samples for each soil order, these findings warrant further confirmation.

Fig. 3

3.4 Impact of soil physicochemical properties on Fe-OC association

Soil texture can potentially influence the accumulation of Fe-bound OC. Figure 4 demonstrates that the fraction of non-calibrated Fe-bound OC showed a significant positive correlation vs. fraction of sand ($r=0.72, p<0.001$), and negative correlations vs. fraction of silt ($r=-0.697, p<0.001$) and fraction of clay ($r=-0.616, p<0.001$). There were similar correlations between
labile OC, and the fraction of sand ($r=0.72$, $p<0.001$), silt ($r=0.72$, $p<0.001$) and clay ($r=0.72$, $p<0.001$). However, the calibrated Fe-bound OC had no significant correlation vs. any of the texture fractions. These correlations indicate that the labile OC was mainly associated with the sand component of forest soils, but that the soil texture did not affect the Fe-bound OC. There has been debate on the relative roles of sand, clay and silt in the stabilization of OC in soil (Percival et al., 2000; Six et al., 2002; Eusterhues et al., 2005; Vogel et al., 2014). Eusterhues et al. (2005) found a relationship between the resistance of organic matter to oxidative degradation and the clay concentration in soils, suggesting the importance of clay minerals in the stabilization and accumulation of soil OC. In contrast, Percival et al. (2000) found that the clay mineral fraction explained little of the variation in the accumulation of OC across a range of soil types in New Zealand. Vogel et al. (2014) found that less than 20% of clay mineral surfaces were covered by the sorption of OC, indicating that a limited proportion of clay mineral surface contributed towards the stabilization of OC. Our results suggest that the Fe oxide-mediated stabilization of OC was not related to the size/aggregation-based process, although the labile carbon concentrations increased with the fraction of sand in the soils.

Fig. 4

The Fe-OC association can also be influenced by the soil pH, which affects the mineral phases of Fe oxides and their surface charge, and their interactions with OC. For our soil samples, the soil pH ranged from 4.1 to 6.3, similar to measurements by Wagai and Mayer (2007) for North America soils. There was no significant correlation between the $f_{\text{Fe-OC}}$ and soil pH, e.g. the HL (Maine) soil with pH of 4.4 had the highest $f_{\text{Fe-OC}}$ of 57.8%, while the TS(II) (Washington) soil with a similar pH of 4.5 only had a $f_{\text{Fe-OC}}$ of 7.4%. For soils with pH ranging from 4.9 to 5.8, $f_{\text{Fe-OC}}$ did not change correspondingly. Contrastingly, values of OC:Fe molar ratios were significantly influenced by the soil pH; expect for one outlier sample of TS(II) (Washington) soil, there was a significant negative correlation between the OC:Fe molar ratio and soil pH ($r=-0.477$, $p=0.09$) (Supplementary Material, Fig. S2). This may be due to the lower pH values favoring the complexation and precipitation of Fe with OC, while higher pH favors sorptive interactions between Fe minerals and OC (Tipping et al., 2002). If comparing samples with similar pH, the soils with higher TOC had higher OC:Fe molar ratios, e.g. the GS soil (TOC = 1.1%) with pH of
4.7 had an OC:Fe molar ratio = 8.5, while the HT (Michigan) soil (TOC = 3.0%) with similar pH of 4.7 had an OC:Fe molar ratio = 17.1. This was consistent with the concept model that the form of Fe was dominated by the Fe-OC co-precipitated complex with relatively higher OC supply (Schwertmann et al., 1986).

3.5 Molecular characteristics of Fe-bound OC

The chemical composition of Fe-bound OC can be substantially different from non-Fe-bound OC (Adhikari and Yang, 2015) with broad implications on the C biogeochemical cycles, although such differences so far have received limited attention. We analyzed the difference in chemical composition of Fe-bound OC compared to non-Fe-bound OC using ATR-FTIR analysis (Fig. 5). Overall, there were limited fingerprint peaks for OC, because of the low concentration of TOC and technical challenge for analyzing whole soil particles with FTIR (Calderon et al., 2011; Simonetti et al., 2012). Reeves (2012) demonstrated that FTIR analysis of mineral soils in the ranges of 1600-1750 and 2800-3000 cm\(^{-1}\) only can be used to study OC. Peaks in the range of 500-1200 cm\(^{-1}\) indicate the presence of clay or other Fe/Al minerals (Fig. 5) (Madejova, 2003; Harsh et al., 2002; Parikh et al., 2014), such as kaolinite or montmorillonite at 850-1200 cm\(^{-1}\) (Madejova, 2003). Absorption at 850-1200 cm\(^{-1}\) can also be due to the presence of polysaccharides, but definitive identification of polysaccharides is not possible in the presence of minerals (Senesi et al., 2003; Tandy et al., 2010). The spectra in the range of 1600-1750 cm\(^{-1}\) normally contain fingerprint peaks for functional groups of amides, carboxylates and aromatics (Parikh et al., 2014), but we did not detect any significant peaks in this range. In the range of 2800-3000 cm\(^{-1}\), there were no significant peaks for the original soil samples, but after Fe extraction we detected significant peaks at 2850 and 2930 cm\(^{-1}\), which are characteristic for the presence of aliphatic carbon. The substantial differences in spectra before and after Fe extraction indicate that aliphatic OC was enriched in the residual soils after extraction. Other functional groups, such as aromatic carbon and hydrophilic functional groups, were more strongly associated with Fe minerals and removed during the Fe extraction, as hydrophilic functional groups can form inner-sphere coordination complexation with iron oxides, and aromatic carbon has electron donor-acceptor interactions with iron oxides (Gu et al., 1995; Axe and Persson, 2001).

Fig. 5
Furthermore, we analyzed the C 1s NEXAFS spectra of two original, non-extracted soils with the highest and lowest values of \( f_{\text{Fe-OC}} \), i.e. HL (Maine) \( (f_{\text{Fe-OC}}=57.8\%) \) and OR (Tennessee) \( (f_{\text{Fe-OC}} \text{ non-detectable}) \) (Supplementary Material, Fig. S3). Three major fingerprint peaks were detected for both soils, including peaks at 285.3, 287.0 and 288.7 eV, which are corresponding to aromatic carbon, aliphatic carbon and carboxylic carbon, respectively (Schumacher et al., 2005; Solomon et al., 2005; Lehmann et al., 2008). The OR (Tennessee) soil had a more substantial signal at 287.0 eV than the HL (Maine) soil, indicating a higher aliphatic carbon concentration in the OR (Tennessee) soil compared to the HL (Maine) soil. Ratio of carboxylic carbon to aromatic carbon (peak height) was 3.8 for HL (Maine) and 1.0 for OR (Tennessee), suggesting that the HL (Maine) soil with higher \( f_{\text{Fe-OC}} \) has relatively more carboxylic carbon compared to aromatic carbon. Hence, the C1s NEXAFS spectra suggest that the soil with the higher \( f_{\text{Fe-OC}} \) has higher concentration of carboxylic C, while the soil with the lower \( f_{\text{Fe-OC}} \) value has a higher aliphatic C concentration. This result is consistent with the comparison of ATR-FTIR spectra in soils before and after Fe extraction, providing evidence that Fe oxides are mainly associated with more hydrophilic and carboxylic carbon, while non-Fe-bound OC was more aliphatic.

To further investigate the relationships between soil OC and Fe minerals, we analyzed the stable C isotopic compositions (\( \delta^{13}\text{C} \)) of Fe-bound vs. non-Fe-bound OC (i.e., the residual OC after DCB extraction). The \( \delta^{13}\text{C} \) for original soil samples ranged from \(-24.5\)‰ to \(-27.5\)‰, and the values for non-Fe-bound OC were \(-25.1\)‰ to \(-28.0\)‰. The \( \delta^{13}\text{C} \) for Fe-bound OC was calculated by combined isotope-mass balance (equation (1))

\[
\delta^{13}\text{C}_{\text{TOC}}\times\text{TOC}=\delta^{13}\text{C}_{\text{labile}}\times\text{OC}_{\text{labile}} + \delta^{13}\text{C}_{\text{Fe-OC}}\times\text{OC}_{\text{Fe}} + \delta^{13}\text{C}_{\text{non-Fe-OC}}\times\text{OC}_{\text{non-Fe}}
\]

(1)

where TOC is the concentration of total organic carbon, \( \text{OC}_{\text{labile}} \) is the concentration of labile OC (extractable by bicarbonate buffer), \( \text{OC}_{\text{non-Fe}} \) is the concentration of non-Fe-bound OC (residual OC after Fe extraction), and \( \text{OC}_{\text{Fe}} \) is the concentration of Fe-bound OC (excluded the labile OC); \( \delta^{13}\text{C}_{\text{TOC}} \) is \( \delta^{13}\text{C} \) for bulk OC, \( \delta^{13}\text{C}_{\text{labile}} \) is \( \delta^{13}\text{C} \) for labile OC, \( \delta^{13}\text{C}_{\text{Fe-OC}} \) is \( \delta^{13}\text{C} \) for Fe-bound OC, and \( \delta^{13}\text{C}_{\text{non-Fe-OC}} \) is \( \delta^{13}\text{C} \) for non-Fe-bound OC. However, it is difficult to directly resolve the \( \delta^{13}\text{C}_{\text{labile}} \) and \( \delta^{13}\text{C}_{\text{Fe-OC}} \) using this equation. We simplified it to equation (2):

\[
\delta^{13}\text{C}_{\text{Fe-OC}}=\frac{(\delta^{13}\text{C}_{\text{TOC}}\times\text{TOC} - \delta^{13}\text{C}_{\text{non-Fe-OC}}\times\text{OC}_{\text{non-Fe}})}{\text{OC}_{\text{Fe}}}
\]

(2)
where \( \delta^{13}\text{C}_{\text{Fe-OC}} \) is \( \delta^{13}\text{C} \) for Fe-bound OC (including the labile OC), \( \delta^{13}\text{C}_{\text{TOC}} \) is \( \delta^{13}\text{C} \) for bulk OC, \( \delta^{13}\text{C}_{\text{non-Fe-OC}} \) is \( \delta^{13}\text{C} \) for non-Fe-bound OC, TOC is the concentration of total organic carbon, \( \text{OC}_{\text{non-Fe}} \) is the concentration of non-Fe-bound OC, and \( \text{OC}_{\text{Fe}} \) is the concentration of Fe-bound OC. The \( \delta^{13}\text{C} \) for Fe-bound OC was heaviest for the TKF (California) soil with a value of \(-23.0\%\), and the lightest for the GS (Florida) forest at \(-27.0\%\). Across all study sites, Fe-bound OC was relatively enriched in \( ^{13}\text{C} \) (1.5±1.2‰ heavier) compared to the non-Fe-bound OC. However, there is also a contribution of labile OC to the Fe-bound OC, where labile OC is the OC extracted during the dithionite-absent extraction described earlier). The \( \delta^{13}\text{C} \) value for labile OC can be calculated using equation (3):

\[
\delta^{13}\text{C}_{\text{labile}} = \frac{\delta^{13}\text{C}_{\text{TOC}} \times \text{TOC} - \delta^{13}\text{C}_{\text{non-labile}} \times \text{OC}_{\text{non-labile}}}{\text{OC}_{\text{labile}}}
\]

where \( \delta^{13}\text{C}_{\text{labile}} \) is \( \delta^{13}\text{C} \) for labile OC, \( \delta^{13}\text{C}_{\text{TOC}} \) is \( \delta^{13}\text{C} \) for bulk OC, \( \delta^{13}\text{C}_{\text{non-labile}} \) is \( \delta^{13}\text{C} \) for non-labile OC, \( \text{OC}_{\text{non-labile}} \) is the concentration of non-labile OC, and \( \text{OC}_{\text{labile}} \) is the concentration of labile OC. Calculated values of \( \delta^{13}\text{C}_{\text{labile}} \) range from -23.4% to -30.3%, and were lighter than the values for \( \delta^{13}\text{C}_{\text{Fe-OC}} \). Although it is not reliable to quantitatively calculate the \( \delta^{13}\text{C} \) for Fe-bound OC subtracting the influences of labile OC, these results indicate that the true value for \( \delta^{13}\text{C}_{\text{Fe-OC}} \) should be even somewhat heavier than the results presented in Fig. 6.

Our results demonstrate that Fe-bound OC was enriched in \( ^{13}\text{C} \) compared to the non-Fe-bound OC in forest soils, which is consistent with results for sediments, where Fe-bound OC was 1.7±2.8‰ heavier than non-Fe-bound OC (Lalonde et al., 2012) (Fig. 6A). Wang et al. (1998) have shown that \(^{13}\text{C}\)-enriched organic matter in sediments was enriched with O and N (due to the presence of compounds such as proteins and carbohydrate groups), while the lipid fraction was relatively \(^{13}\text{C}\)-depleted. Similarly, compound-specific isotopic analyses have shown that O/N-rich constituents, such as cellulose, hemi-cellulose and amino acids, are \(^{13}\text{C}\)-enriched compared to hydrocarbons (Glaser, 2005), and these \(^{13}\text{C}\)-enriched O/N-rich compounds can associate with Fe oxide extensively through inner-sphere coordination interactions (Parikh et al., 2014). The value of \( \Delta^{13}\text{FeOC}_{\text{non-FeOC}} = \delta^{13}\text{C}_{\text{Fe-OC}} - \delta^{13}\text{C}_{\text{non-Fe-OC}} \) (difference in \( \delta^{13}\text{C} \) for Fe-bound OC and non-Fe-bound OC) was inversely correlated with the molar ratio of OC:Fe (\( r=-0.53, p=0.05 \), Fig. 6B). These relationships suggest that the enrichment in \( ^{13}\text{C} \) was to some degree related to the OC:Fe ratio, with lower OC:Fe leading to higher enrichment in \( ^{13}\text{C} \). As discussed previously, lower OC:Fe ratios indicate an increased contribution from sorptive interactions of OC with Fe minerals as compared to incorporation of OC within iron oxides and OC, and these sorptive interactions...
between O/N-rich organic compounds and Fe oxide results in the enrichment of $^{13}$C of Fe-bound OC vs. non-Fe-bound OC. Previous studies have attributed the stability of relatively labile and reactive compounds, such as amino acids and sugars, to their interactions with minerals (Schmidt et al., 2011), and our results demonstrated the importance of sorption to Fe minerals in increasing the stability of relatively reactive labile compounds.

Fig. 6

Nitrogen (N)-containing functional groups are potentially important for the association between OC and Fe oxides, although the concentrations of N are much lower than C. The bulk soil contained 0.05-0.45 % N, while the non-Fe-bound component (i.e. the residual solid after DCB extraction) contained 0.06-0.32 % N. Concentrations of Fe-bound N, calculated by difference, ranged up to 0.13 %. However, it is important to note that this number is based without a calibration for labile N that may be removed by the dithionite-free DCB extraction (data not available). There were significant correlations between C and N concentrations for both bulk soils ($r=0.847$, $p<0.001$: Supplementary Material, Fig. S4) and the non-Fe-bound residual components ($r=0.858$, $p<0.001$: Supplementary Material, Fig. S4), with molar C/N ratios of 14.2±2.6 and 13.7±2.3 for bulk and non-Fe-bound OC, respectively. These C/N values are essentially identical to a previously observed molar C/N ratio = 14.3 for a large set of world-wide soils samples (Cleveland et al., 2007), and a molar C/N ratio = 14.4 for OC-rich samples in China (Tian et al., 2010). This result suggests that C/N ratios for Fe-bound OC did not differ from that of non-Fe-bound OC, assuming that the labile carbon did not have a substantially different C/N ratio. Therefore, in contrast to the $^{13}$C enrichment observed for Fe-bound OC, the interactions with Fe minerals did not affect the C/N ratio substantially.

4. Conclusion

Fe oxides are recognized as an important mineral phase regulating the amount and characteristics of OM in forest soils. The spatial variability of Fe-bound OC is governed by the geographical factors, such as latitude and annual mean temperature, and also the soil physicochemical properties.
Chemical composition of Fe-bound OC is substantially different from the rest compartment of soil OC. Overall, this study provided a comprehensive investigation into the spatial variance of Fe-bound OC in forest soils, its governing factors, and how the Fe-OC associations affect the chemical characteristics of OC. As a substantial amount of OC was bound to Fe oxides, and association with Fe affected the quality of OC, Fe oxides can regulate the biogeochemical cycles of carbon and its response to climate change.

Acknowledgements

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Figure Captions

Figure 1. Concentrations of total carbon (TC), total organic carbon (TOC) and Fe-bound OC in 14 forest soils across the United States. Duplicate measurements were conducted for each of two plots in every forest site. Error bars represent standard deviation of measurements of four replicates for each forest site.

Figure 2. A Concentration of reactive Fe and OC:Fe molar ratio in US forest soils. B Relationship between the fraction of Fe-bound OC in TOC ($f_{Fe-OC}$/ OC:Fe molar ratio and reactive Fe concentration in US forest soils.

Figure 3. Correlation between the TOC, reactive Fe, concentration of Fe-bound OC, $f_{Fe-TOC}$, OC:Fe and ecogeographical parameters including latitude, longitude, elevation (asl), precipitation (mean annual) and temperature (annual mean).

Figure 4. Correlation of the fractions of iron-bound organic carbon (uncalibrated and calibrated for loss of labile OC) and labile organic carbon vs. fractions of sand, silt, and clay in forest soils.

Figure 5. Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) analysis for representative forest soils before (black line) and after Fe extraction (red line). All the spectra are background-calibrated. Among the 14 forest soils sampled in this study, we used five different forest soils, with $f_{Fe-OC}$ ranging 5.6-57.8%.

Figure 6. A. $\delta^{13}$C of total organic carbon and non-iron bound organic carbon for 14 U.S. forest sites. B. Correlation between $\Delta^{13}$FeOC-nonFeOC and molar ratio of OC:Fe.
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<tr>
<th>Forest ID</th>
<th>Abb. r.</th>
<th>Location</th>
<th>Soil Order</th>
<th>Climate Zone</th>
<th>Precip. a (mm y⁻¹)</th>
<th>Temp b (°C)</th>
<th>LAT c (°)</th>
<th>LONG d (°)</th>
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<td>9.8</td>
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</table>

a: annual precipitation; b: annual mean temperature; c: latitude; d: longitude.
Fig. 1
Fig. 2

A

Reactive iron content (mg/g)

OC:Fe Ratio

B

Fraction of iron-bound OC (%)

OC:Fe Ratio

Reactive Fe content (mg/g)
Fig. 3

- TOC (%): $r=0.619$, $p=0.018$
- Reactive Fe (mg g$^{-1}$): $r=0.523$, $p=0.055$
- Fe-bound OC (%): $r=0.525$, $p=0.054$
- OC:Fe

Latitude (˚): -150 to 0
Longitude (˚): -150 to 0
Elevation (m): 0 to 4000
Precipitation (mm): 0 to 2000
Temperature (˚C): 0 to 25

(Values provided for illustration purposes.)
Fig. 4
HL, $f_{Fe-OC}=57.8\%$

TKF, $f_{Fe-OC}=20.0\%$

MS, $f_{Fe-OC}=13.0\%$

LVF, $f_{Fe-OC}=11.0\%$

AL, $f_{Fe-OC}=5.6\%$

Fig. 5
Fig. 6

Difference in δ¹³C (‰ vs. VPDB)

TOC
Non-Fe-bound OC
Fe-bound OC
Labile OC

OC:Fe ratio