July 12, 2016

Responses to Comments

Comments are in black font and our responses are in italicized font.

Associate Editor

Decision: Publish subject to minor revisions (Editor review) (04 Jul 2016) by Dr. Roland Bol

Comments to the Author:
The authors have dealt in an extensive and critical way with the comments posed by the reviewer and the paper this warrant publication. There is a very minor issue to be clarified after which the paper is ready for publication in BG (see below)

Thank you very much for your decision and all the helpful reviews. The manuscript was revised accordingly (see attached). Language of the manuscript was further polished, and minor editorial changes were also marked.

The authors should in their next upload clarify the issue of the mean residence time of C in these forest soils as raised their response to the reviewer on this point is not 100% clear.

Reviewer:

Line 258: I did expect this as these are relatively “old” soils. The long aging process accumulates old and possibly recalcitrant organic matter along with very stable iron minerals… Also, aging results in more stable iron-organic matter associations as shown by Kaiser et al 2007 (doi:10.2136/sssaj2006.0189).

Author response:

We have limited information about aging. But our 14C data indicates the age of soils (AL and TK) ranged from 1993 to 2008 (modern soil) (Zhao, unpublished). We did not observe old soils.

AE comment:

In which year were these soils sampled. You imply that based on the (bomb-)14C content in these forest soil (0-20cm) you estimated that most C had entered and were present in the soils when samples was from between 1993 and 2008 (so if soils were sampled in 2011 the MRT would be 3 to 8 years. Indeed very recent. Clearly there is/was also a discrepancy between what the reviewer expected (older soils) and what was found (very recent soil material) what could be the reason for this?

A good point. The soils were sampled from top layer (0-20 cm) during 2007-2009. At this layer, carbon is refreshed relatively rapidly, and maintains relatively young. Although we agree with the reviewer that OC could be more stable when bound with Fe oxides, we did not demonstrate the difference between the age of Fe-bound OC and the rest of the OC. In the manuscript, we added the sampling time for soils in the method part.
Reviewer #1 (Anonymous Referee #2 Received and published: 19 February 2016)

The authors draw no conclusion from their data. This section needs to be revised. The current section limits itself to general statements and relatively vague summarizing sentences. In lines 309-311 the authors state that the chemical composition of Fe-bound can be substantially different and that this will have broad implications on the C biogeochemical cycles. They then demonstrate that there actually is a difference in chemical composition. The logical consequence hence is to now conclude which implications on the C cycle are to be expected from their findings. "Fe oxides can regulate the biogeochemical cycles of carbon and its response to climate change" is a very general statement and not a novel conclusion. In which way do they influence the C cycle considering that Fe-bound C is more aliphatic? "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." is too general and imprecise. In which way do they influence the spatial variability? How does this reflect on the C cycle?

We will revise the conclusion to be more specific, according to the comments and suggestions:

Overall, this study provided a comprehensive investigation into the amount and characteristics of Fe-bound OC in forest soils as well as the impact of soil physicochemical properties on Fe-bound OC. On average, Fe-bound OC contributed to 37.8% of TOC in forest soils, composing an important component of C cycles in terrestrial ecosystem. The OC:Fe molar ratios in the forest soils studied ranged from 0.56 to 17.7, indicating the importance of both sorptive and incorporative interactions between Fe and OC. Fe-OC increased with latitude, and reached the peak value for soils with an annual mean temperature of 6.6˚C, as a result of the temperature dependence of Fe mineral phase and OC transformation. Combined studies of FTIR, NEXAFS, and 13C analysis revealed that Fe-bound OC was less aliphatic, more carboxylic, and more enriched in 13C, compared to non-Fe-bound OC. Assuming Fe-bound OC is relatively stable, Fe oxides serve as a storage reservoir on decadal time scales for hydrophilic and carboxylic OC, which would be otherwise relatively more available for microbial degradation.

Reviewer #2 (t. riedel thomas.riedel@tu-braunschweig.de)

Zhao et al investigated the role of iron oxides for organic carbon storage and preservation in north american forest soils. The study is based on chemical extractions and subsequent analysis of total iron and carbon. The different carbon fractions have than further been analysed for carbon isotopic as well as molecular composition. The study covers a number of different soils from various climatic regions, which is later considered in the discussion. Overall this is a nice study and the results provide good evidence for the emerging idea that iron minerals are one of many factors that govern organic matter preservation in soils. The study is well written, clearly organized and for the most part technically sound. The results are interesting to readers from soils sciences, biogeochemistry and partly for climate scientists working on soil-climate feedbacks. However,
there a few minor and some major points that I like to see revised before the paper can eventually be published.

Thank you for the positive comments about our manuscript.

Major remarks: 1) I did not check the references of Obrist et al to see how soils were sampled. However, since this is basic information for the present study I suggest that the sampling should briefly be described in the paper. For example, please explain if the different soil horizons were mixed to a composite sample. If not, which horizon was sampled and used in this study? In a spodosol, iron and organic matter are enriched in the deeper horizons, while being depleted in shallow ones. The chemical composition of both likely will be different in the different horizons, which will strongly affect the outcome of this study.

This is a good suggestion. All the soils used in this study are from top layer (0-20 cm).

We will add more information about soil sampling in section 2.2:

More detailed information about the sites and sampling protocols can be found in previous publications (Obrist et al., 2011, 2012, 2015). Briefly, two replicate plots at each forest site were sampled. During 2007-2009, top soils (0-20 cm) from all sites were collected using clean latex gloves and stainless steel sampling equipment. All samples were immediately transferred to plastic freezer bags and kept on ice before transportation to the laboratory.

2) Unfortunately, the analytical section totally misses information on data quality. For the determinations of iron, nitrogen and carbon please add information on accuracy and precision.

We will add the information about accuracy and precision information for analysis toward the end of session 2.3, 2.4 and 2.6:

All analyses are based on standard curves with $R^2 > 0.99$. The detection limit for N is 0.2 mg/g soil. The average coefficient of variation for the analysis of N is 20.5%.
All analyses are based on standard curves with $R^2 > 0.99$. The detection limit for C is 0.2 mg/g soil. The average coefficient of variation for the analysis of C is 20.2%.
All analyses are based on standard curves with $R^2 > 0.99$. The detection limit for Fe is 0.04 mg/g soil. The average coefficient of variation for the analysis of Fe is 25.8%.

3) I wonder if the chemical extractions influenced the molecular properties of the organic matter? Has this been tested by the authors? I suppose that it is possible for dithionite to change the properties oxidized functional groups. What about sulfurization of organic matter? Please provide a small discussion based on literature on the effect of reducing agents on organic matter and how that might possibly affect the results of the present study.

Good point. Previous studies did not note the transformation of organic matter, upon the reaction with dithionite (Lalonde et al., 2012; Wagai and Mayer, 2007). The most possible reaction between dithionite and organic matter is the reduction of oxidized organic functional groups. Our recent study showed that dithionite could reduce quinone groups in natural organic matter (Adhikari et
al., 2016). For our discussion, quinone normally does not contribute significantly to the TOC in mass, and is not the focus of our study. Most likely, other major functional groups, such as carboxylic and carbonyl functional groups, cannot be reduced by dithionite based on their reduction potentials (Bar-Even et al., 2012; Mayhew et al., 1978). Organic sulfur speciation is beyond the scope of this research. Further investigations are needed to elaborate the detailed influences of dithionite reduction on the molecular properties of organic matter. We will add relevant discussion.

Minor remarks: Line 19. Having this number in the abstract somewhat skews the results of this study towards a higher importance of iron minerals than it really is. Fig. 1 gives a totally different picture. Maybe report median or range here.

We will change the statement to:

*We found that on average Fe-bound OC contributed 37.8% of total OC (TOC) in forest soils.*

Lines 90-91: Coarse material was not removed? What about roots?

*Roots and visible plant material were removed. Large particles (>2 mm) were removed by dry sieving. The sentence will be changed accordingly.*

Lines 119-121: Are manganese oxides also affected? Has Mn been measured in addition to Fe?

*Manganese oxides are likely also reduced by dithionite based on their reduction potentials. In soil, generally the Mn content was two orders of magnitude lower than that of Fe (Sparks, 2011). Total electrons, which can be accepted by MnO₂, is 10% of that for iron oxides (Lovley, 2011). OC bound to Mn minerals would be very likely much less than that associated with Fe oxides. In this study, we did not analyze the influences of manganese oxides on the OC stock.*

Lines 130-131: This is basically the ion exchangeable organic carbon. I probably missed it, but why was this done? Was the exchangeable organic carbon subtracted from the chemically extracted carbon? If not, please discuss why.

*Yes. We subtracted the ion exchangeable OC from the DCB-extractable OC, for determination of the Fe-bound OC.*

Line 151: Why was iron only determined at a single wavelength of 259.9 nm? Using multiple wavelengths has the advantage of identifying interferences that might occur on one wavelength, which can than easily be corrected.

*We picked up the wavelength based on the best standard curve established.*

Lines 160-175 and 309-331: I am not an expert on NEXAFS and FTIR. I suggest the authors and the editor to refer to another reviewer with more expertise on these methods.

*We worked with Canadian beamline scientist to ensure the interpretation and discussion for NEXAFS data.*
Line 180: Are numbers reported as one or two standard deviations of replicates? If so, how many (eg, n = 3)? Are this analytical or sampling replicates?

All results are based on duplicated sampling sites.

Lines 187-192: This part is speculative. Since not all soil types are covered the comparison might not hold up at the global level. For example, organic rich soils such as Histosols and Chernozems were not as much considered as they should be based on their global distribution. Further, since the North American distribution of soils differs from the global distribution in that Cambisols, Regosols and iron-rich chromic luvisols are underrepresented, this section should be removed.

We acknowledge limited types of soils were sampled and analyzed in this study. It is still good information for evaluation of carbon cycles on the large scale. We will shorten the discussion to:

Based on an estimate that 1502 Pg (Pg=1×10^15 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.

Lines 202-205: Why was that a surprise? Was it one the main hypothesis of the study? If so, this might be stated in the introduction or methods. After all, shouldn’t non-reactive iron store more organic carbon than reactive iron?

We speculated the fraction of Fe-bound OC would be correlated to the amount of reactive Fe, DCB extractable Fe or free Fe oxides. To minimize confusion, we will revise the statement:

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.

Line 214: How do you define “most of the”? It might be better to state “2/3 of the” or some equally (quantitative) phrase.

We will change “most of the” to “two thirds of the”.

Lines 241-262: Climate actually controls the type of soil and the type of iron minerals directly through controlling the weathering of iron-bearing primary minerals and the formation of secondary iron-phases. So 6.6°C is probably the ideal condition for soil organic matter production and the formation OM-binding FeOOH minerals. I like to hear a statement by the authors on why “Fe-OC interactions may be particularly pronounced” at 6.6 °C. Can there really be a temperature control on this type of interaction? If so, please provide references for this.

Very insightful suggestion. We will add discussion:

Temperature dependence of Fe-bound OC was regulated by the effects of temperature on the mineral phase of Fe oxides and OC dynamics. Given that ferrihydrite can incorporate more OC
than other crystalline Fe oxides, an increase in temperature favors the transformation of ferrihydrite to other crystalline iron oxides (Gnanaprakash et al., 2007; Zhao et al., 1994). However, increase in temperature can also accelerate weathering of other minerals, and increased release of silicon can retard the transformation of ferrihydrite (Cornell et al., 1987; White and Blum, 1995). However, there is also evidence that temperature can affect the chemical composition of soil OC substantially (Conant et al., 2011). For example, increased temperature decreased the content of oxidized functional groups, such as saccharides, which would consequently inhibit the interactions between OC and Fe oxides (Amelung et al., 1997). The overall pattern can be resulted from combined effects of temperature on Fe mineral phase and OC transformation. Further investigations are required to elucidate the mechanism more accurately.

Lines 252-254: This sentence is somewhat unclear. How does temperature increase the amount of iron bound organic matter (see comment above)? Please re-write.

The sentence will be re-phrased to:
The concentration of Fe-bound OC and $f_{Fe-OC}$ reached peak value at 6.6°C.

Line 258: I did expect this as these are relatively “old” soils. The long aging process accumulates old and possibly recalcitrant organic matter along with very stable iron minerals… Also, aging results in more stable iron-organic matter associations as shown by Kaiser et al 2007 (doi:10.2136/sssaj2006.0189).

We have limited information about aging. But our $^{14}$C data indicates the age of soils (AL and TK) ranged from 1993 to 2008 (modern soil) (Zhao, unpublished). We did not observe old soils.


Good suggestion. We will revise the discussion to:
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. Reduced chemical potential of soil organic matter in small pores of clay-rich soils also limits its microbial degradation and enhance its stabilization (Riedel and Weber, 2016). 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.

Lines 304-306: This short sentence is unclear. Please explain this ‘concept model’ in more detail.

We will revise the statement and explain the concept model more clearly:
This is consistent with Schwertmann et al.1986, who found that the major form of Fe would change from FeOx to complexes with OC when there is higher OC supply.
A nice result. We have observed a similar effect using a complementary method (ultra-high resolution mass spectrometry) in a recent study (doi:10.1016/j.orggeochem.2014.02.003). During a leaching test a soil column had turned anoxic and the reductive dissolution of iron- and manganese oxides lead to an increase in the abundance of aromatic compounds leaching from the soil columns. Besides polyphenolic compounds we also observed a release of iron-bound condensed aromatics leading me to belief that iron minerals probably play an important role in sequestering 'black carbon'-type compounds in soils. Do the NEXAFS or FTIR data of this study allow identification of this type of organic matter?

Very interesting results. We are planning to measure the Fe-bound black carbon in these forest soils, using the quantification of thermo analysis. From NEXAFS and FTIR, it is challenging to unambiguously identify the contribution of black carbon. We will add relevant discussion after line 331:

This result was consistent with a previous study using ultra-high resolution mass spectrometry, showing the release of more aromatic carbon during the reductive dissolution of Fe oxides (Riedel et al., 2014).

Lines 385 and 387. “O/N-rich”. This is written like a ratio, but that is not meant here, right?

We meant rich in both O and N. We will change it to “O- and N-rich”

Lines 382-385. This probably indicates that the lipids are derived from microbial biomass which usually has a lighter isotopic value.

Good point. We will revise the discussion to:

Previous studies shown that $^{13}$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 microbial biomass-derived lipid fraction was relatively $^{13}$C-depleted (Wang et al., 1998; Zelles et al., 1992).

Lines 391-392: But it could also be vice versa. Suppose compounds with heavier isotopic composition preferentially sorb to iron.

Yes. It is difficult to determine the driving force. We will revise the statement to:

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

Lines 392: Where was this “discussed previously”?

We will change it to:

As discussed previously (session 3.2)

Lines 403-404. A reference would be good here.
We will add citation of (Yang et al., 2012; Barber et al., 2014).


Table 1: I had a hard time understanding table 1, because I am not familiar with the American soil taxonomy. In order to make the paper more readable to a readership beyond North America it might be a good idea if the authors could change to the international FAO soil classification standard, if this is possible. After all the paper has been submitted to a journal run by the ‘European’ Geophysical Union…

We will add FAO classification.

Figure 3: What was the hypothesis behind the linear regression analysis of TOC vs latitude? Did the authors suspect a linear relationship? If so, please explain why.

We have relevant discussion in the text (line 235-237):
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). We only discussed the correlation, rather than regression. To avoid confusion, we will remove the line.
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<th>Forest ID</th>
<th>Abb r.</th>
<th>Location</th>
<th>Soil Order (US)</th>
<th>Soil Class (FAO)</th>
<th>Climate Zone</th>
<th>Precip. (mm y(^{-1}))</th>
<th>Temp (°C)</th>
<th>LAT(°)</th>
<th>LONG(°)</th>
<th>Elevation (m asl)</th>
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<td>Luvisols &amp; Greyzems</td>
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<td>Podzols &amp; Lithosols</td>
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<td>OR</td>
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<td>Cambisols</td>
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<td>1140</td>
<td>9.8</td>
<td>47.38</td>
<td>-121.93</td>
<td>220</td>
</tr>
</tbody>
</table>

a: Food and Agriculture Organization; b: annual precipitation; c: annual mean temperature; d latitude; e: longitude.
References:


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 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. On average, Fe-bound OC contributed 37.8% 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 the ratios indicate the importance of both sorptive and incorporative interactions. The fraction of Fe-bound OC in TOC ($f_{\text{Fe-OC}}$) 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 $f_{\text{Fe-OC}}$ increased with 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 $^{13}\text{C}$ compared to the non-Fe-bound OC, but C/N ratios did not differ substantially. In summary, $^{13}\text{C}$-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 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 an 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, the factors that control Fe-bound OC concentrations, and the characteristics of Fe-bound OC with respect to the 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 vs. non-Fe-bound OC, including how Fe-OC association influenced the chemical properties of OC and the 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). Briefly, two replicate plots at each forest site were
sampled. During 2007-2009, top soils (0-20 cm) from all sites were collected using clean latex gloves and stainless steel sampling equipment. All the samples were immediately transferred to plastic freezer bags and kept on ice before transportation to the laboratory. 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 after the removal of roots and visible plant material and large particles (>2 mm) by dry sieving.

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. All analyses are based on standard curves with \( R^2 > 0.99 \). The detection limit for C is 0.2 mg g\(^{-1}\) soil. The average coefficient of variation for the analysis of C is 20.2%.

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. All analyses are based on standard curves with \( R^2 > 0.99 \). The detection limit for N is 0.2 mg g\(^{-1}\) soil. The average coefficient of variation for the analysis of N is 20.5%.

**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 \(^{\delta^{13}}\)C composition. The mass of residual particles was 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. All analyses are based on standard curves with $R^2$>0.99. The detection limit for Fe is 0.04 mg g$^{-1}$ soil. The average coefficient of variation for the analysis of Fe is 25.8%.

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, \( I_0 \) was collected by measuring the scatter of the incident beam from a freshly Au-coated Si wafer using SDD. Before the \( I_0 \) 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 forest soils (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=1×10^{15} g) of TOC 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.

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 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 site MS (California) had the highest concentration of reactive Fe, while
a Spodosol in forest site GS (Florida) had the lowest reactive Fe concentration. **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 about two thirds 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 almost 20 (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 a maximum value of 17.8 (Fig. 2A), implying that incorporation of OC into Fe oxides dominated at these sites. **Similar to** $f_{Fe-OC}$, **OC:Fe ratios were not related to the concentration of reactive Fe and showed large variation for soils with similar concentration of total reactive Fe (Fig. 2B). This further indicates that** 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 oxides have 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 $f_{Fe-OC}$ 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 forest soils based on the Fe concentrations in soils alone.
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). The concentration of reactive Fe, if excluding soil MS in California, was also significantly related to latitude ($p=0.824$, $r=0.001$). Both concentrations of Fe-bound OC and $f_{Fe-Oc}$ were also correlated with latitude ($p=0.523$, $r=0.053$; $p=0.525$, $r=0.054$). Among our samples, the soil in forest HL in Maine, one of the three northern-most site 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 $f_{Fe-Oc}$ was 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). The concentration of Fe-bound OC and $f_{Fe-Oc}$ reached peak value with mean annual temperatures at 6.6°C, with lower values both at higher and lower temperatures. Temperature dependence of Fe-bound OC can be regulated by effects of temperature on the mineral phase of Fe oxides and OC dynamics. Given that ferrihydrite can incorporate more OC than other crystalline Fe oxides, an increase in temperature favors the transformation of ferrihydrite to other crystalline iron oxides (Gnanaprakash et al., 2007; Zhao et al., 1994). However, an increase in temperature can also accelerate weathering of other minerals, and increased release of silicon can slow the transformation of ferrihydrite (Cornell et al., 1987; White and Blum, 1995). However, there is also evidence that temperature can affect the chemical composition of soil OC substantially (Conant et al., 2011). For example, increased temperature decreased the content of oxidized...
functional groups, such as saccharides, which would consequently inhibit the interactions
between OC and Fe oxides (Amelung et al., 1997). The overall pattern can result from combined
effects of temperature on Fe mineral phase and OC transformation. Further investigations are
required to elucidate the mechanism more accurately. 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, the highest concentration of Fe-bound OC were observed in Spodosols.
Regarding \( f_{\text{Fe-OC}} \), the highest values were also found 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% of 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 with the fraction of sand \((r=0.72, \ p<0.001)\), and negative correlations with the
fraction of silt \((r=-0.697, \ p<0.001)\) and clay \((r=-0.616, \ p<0.001)\). There were similar positive
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
with 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 is a 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. Reduced chemical potential of soil
organic matter in small pores of clay-rich soils also limits microbial degradation and enhance its
stabilization (Riedel and Weber, 2016). 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; except 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 Schwertmann et al. (1986), who found that the major form of Fe would change from FeO$_x$ to complexes with OC when there is higher OC supply.

### 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). This result was consistent with a previous study using ultra-high resolution mass spectrometry, showing the release of more aromatic carbon during the reductive dissolution of Fe oxides (Riedel et al., 2014). Analysis for the chemical nature of Fe-bound OC can be influenced by the potential reaction of natural organic matter with dithionite, which was not noticed in previous studies (Lalonde et al., 2012; Wagai and Mayer, 2007). The most likely reaction between dithionite and organic matter is the reduction of oxidized organic functional groups. Our recent study showed that dithionite could reduce quinone groups in natural organic matter (Adhikari et al., 2016). Most likely, other major functional groups, such as carboxylic and carbonyl functional groups, cannot be reduced by dithionite based on their reduction potentials (Bar-Even et al., 2012; Mayhew et al., 1978). Further investigations are needed to elaborate the detailed influences of dithionite reduction on the molecular properties of organic matter.
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) ($f_{\text{Fe-OC}}$ 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 ($\delta^{13}C$) of Fe-bound vs. non-Fe-bound OC (i.e., the residual OC after DCB extraction). The $\delta^{13}C$ 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 $\delta^{13}C$ for Fe-bound OC was calculated by combined isotope-mass balance (equation (1))

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

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

(2)

where \(\delta^{13}C_{\text{Fe-OC}}\) is \(\delta^{13}C\) for Fe-bound OC (including the labile OC), \(\delta^{13}C_{\text{TOC}}\) is \(\delta^{13}C\) for bulk OC, \(\delta^{13}C_{\text{non-Fe-OC}}\) is \(\delta^{13}C\) 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 \(\delta^{13}C\) 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 \(^{13}C\) (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-extraction described earlier. The \(\delta^{13}C\) value for labile OC can be calculated using equation (3):

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

(3)

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

Our results demonstrate that Fe-bound OC was enriched in \(^{13}C\) 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). Previous studies showed that \(^{13}C\)-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 microbial biomass-derived lipid fraction was relatively \(^{13}C\)-depleted (Wang et al., 1998; Zelles et al., 1992). Similarly, compound-specific isotopic analyses have shown that oxygen- and nitrogen-rich constituents, such as cellulose, hemi-cellulose and amino acids, are \(^{13}C\)-enriched compared to hydrocarbons (Glaser, 2005), and these \(^{13}C\)-enriched oxygen- and nitrogen-rich compounds can associate with Fe oxide extensively through inner-sphere coordination interactions (Parikh et al., 2014). The value of \(\Delta^{13}C_{\text{FeOC-NonFeOC}} = \delta^{13}C_{\text{Fe-OC}} - \delta^{13}C_{\text{non-Fe-OC}}\) (difference in \(\delta^{13}C\) 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 \(^{13}C\) was to some degree
related to the OC:Fe ratio. As discussed previously (section 3.2), 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 oxygen- and nitrogen-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 (Yang et al., 2012; Barber et al., 2014). 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 $14.2\pm2.6$ and $13.7\pm2.3$ 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
Overall, this study provided a comprehensive investigation into the amount and characteristics of Fe-bound OC in forest soils as well as the impact of soil physicochemical properties on Fe-bound OC. On average, Fe-bound OC contributed to 37.8% of TOC in forest soils, composing an important component of C cycles in terrestrial ecosystem. The OC:Fe molar ratios in the forest soils studied ranged from 0.56 to 17.7, indicating the importance of both sorptive and incorporative interactions between Fe and OC. $f_{\text{Fe-OC}}$ increased with latitude, and reached the peak value for soils with an annual mean temperature of 6.6°C, as a result of the temperature dependence of Fe mineral phase and OC transformation. Combined studies of FTIR, NEXAFS, and $^{13}$C analysis revealed that Fe-bound OC was less aliphatic, more carboxylic, and more enriched in $^{13}$C, compared to non-Fe-bound OC. Assuming Fe-bound OC is relatively stable, Fe oxides serve as a storage reservoir on decadal time scales for hydrophilic and carboxylic OC, which would be otherwise relatively more available for microbial degradation.

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**References**


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.
<table>
<thead>
<tr>
<th>Forest ID</th>
<th>Abbr.</th>
<th>Location</th>
<th>Soil Order (US)</th>
<th>Soil Class(^a) (FAO)</th>
<th>Climate Zone</th>
<th>Precip.(^b) (mm y(^{-1}))</th>
<th>Temp(^c) (°C)</th>
<th>LAT(^d) (°)</th>
<th>LONG(^e) (°)</th>
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</table>

\(^a\): Food and Agriculture Organization; \(^b\): annual precipitation; \(^c\): annual mean temperature; \(^d\): latitude; \(^e\): longitude.
Fig. 1

- Thompson Forest I, WA
- Truckee, CA (post-fire)
- Marysville, CA
- Little Valley, NV (post-fire)
- Niwot Ridge, CO
- Ashland, MO
- Oak Ridge, TN
- Bartlett, NH
- Howland, ME

Legend:
- Total Carbon Content
- Total Organic Carbon Content
- Calibrated Iron-bound OC content

Scale bar:
- 3%
- 2%
- 1%
- 0%

0%
Fig. 2

A. Reactive iron content (mg g\(^{-1}\))

B. Fraction of iron-bound OC (%)
Fig. 3
Fig. 4
Fig. 5

HL, $f_{\text{Fe-OC}}=57.8\%$

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

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

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

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

Wavenumber (cm$^{-1}$)
Fig. 6