

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 But our ¹⁴C data indicates the age of soils (AL and TK) ranged from 1993 to 2008 (modern soil) (Zhao, unpublished).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. $f_{\text{Fe-OC}}$ 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.

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 then 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 ($Pg=1 \times 10^{15}$ 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 $f_{\text{Fe-OC}}$ 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.

Lines 282-284: ...and maybe the reduced chemical potential in small pores of clay-rich soils also limits microbial degradation of soils organic matter (Riedel & Weber 2016, doi:10.2136/sssaj2015.02.0085).

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.

Lines 347-349: 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).

Yang, W. H., Weber, K. A., and Silver, W. L.: Nitrogen loss from soil through anaerobic ammonium oxidation coupled to iron reduction. Nature Geosci., 5, 538-541, 2012.

Barber, A., Lalonde, K., Mucci, A., and Gélinas, Y.: The role of iron in the diagenesis of organic carbon and nitrogen in sediments: a long-term incubation experiment. Mar. Chem., 162, 1-9, 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.

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

| Forest ID | Abb. r. | Location | Soil Order (US) | Soil Class ^a (FAO) | Climate Zone | Precip. ^b (mm y ⁻¹) | Temp ^c (°C) | LAT(°) ^d | LONG(°) ^e | Elevation (m asl) |
|---------------------------|-------------|-------------------------|-----------------|-------------------------------|-----------------------|--|------------------------|---------------------|----------------------|-------------------|
| Ashland | AL | Ashland, Missouri | Alfisols | Luvisols & Greyzems | Humid Continental | 1023 | 13.9 | 38.73 | -92.20 | 210 |
| Bartlett | BL | Bartlett, New Hampshire | Spodosols | Podzols & Lithosols | Humid Continental | 1300 | 4.5 | 44.0 | -71.29 | 272 |
| Marysville | MS | Marysville, California | Mollisols | Luvisols | Mediterranean climate | 775 | 16.9 | 39.25 | -121.28 | 386 |
| Gainesville | GS | Gainesville, Florida | Spodosols | Podzols | Humid Subtropical | 1228 | 21.7 | 29.74 | -82.22 | 50 |
| Oak Ridge | OR | Oak Ridge, Tennessee | Ultisols | Acrisols | Humid Subtropical | 1350 | 14.5 | 35.97 | -84.28 | |
| Little Valley (post-fire) | LVF | Little Valley, Nevada | Entisols | Arenosols | Highland Climate | 551 | 5.0 | 39.12 | -119.93 | 2010 |
| Little Valley | LV | Little Valley, Nevada | Entisols | Arenosols | Highland Climate | 550 | 5.0 | 39.12 | -119.93 | 2011 |
| Truckee (post-fire) | TKF | Truckee, California | Alfisols | Luvisols | Highland Climate | 569 | 6.0 | 39.37 | -120.1 | 1768 |
| Truckee | TK | Truckee, California | Alfisols | Luvisols | Highland Climate | 568 | 5.9 | 39.37 | -120.1 | 1767 |
| Niwot Ridge | NR | Niwot Ridge, Colorado | Alfisols | Cambisols | Highland Climate | 800 | 1.3 | 40.03 | -105.55 | 3050 |
| Hart | HT | Hart, Michigan | Spodosols | Podzols | Humid Continental | 812 | 7.6 | 43.67 | -86.15 | 210 |
| Howland | HL | Howland, Maine | Spodosols | Luvisols | Humid Continental | 1040 | 6.7 | 45.20 | -68.74 | 60 |
| Thompson I | TSI | Ravensdale, Washington | Inceptisols | Cambisols | Highland Climate | 1141 | 9.8 | 47.38 | -121.93 | 221 |
| Thompson II | TSII | Ravensdale, Washington | Inceptisols | Cambisols | Highland Climate | 1140 | 9.8 | 47.38 | -121.93 | 220 |

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

References:

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1 Iron-Bound Organic Carbon in Forest Soils: Quantification 2 and Characterization

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11 12 **ABSTRACT**

13 Iron oxide minerals play an important role in stabilizing organic carbon (OC) and regulating the
14 biogeochemical cycles of OC on the earth surface. To predict the fate of OC, it is essential to
15 understand the amount, spatial variability, and characteristics of Fe-bound OC in natural soils. In
16 this study, we investigated the concentrations and characteristics of Fe-bound OC in soils
17 collected from 14 forests in the United States, and determined the impact of ecogeographical
18 variables and soil physicochemical properties on the association of OC and Fe minerals. **On**
19 **average, Fe-bound OC contributed 37.8% of total OC (TOC) in forest soils.** Atomic ratios of
20 OC:Fe ranged from 0.56 to 17.7 with values of 1-10 for most samples, and the ratios indicate the
21 importance of both sorptive and incorporative interactions. The fraction of Fe-bound OC in TOC
22 ($f_{\text{Fe-OC}}$) was not related to the concentration of reactive Fe, which suggests that the importance of
23 association with Fe in OC accumulation was not governed by the concentration of reactive Fe.
24 Concentrations of Fe-bound OC and $f_{\text{Fe-OC}}$ increased with latitude and reached peak values at a
25 site with a mean annual temperature of 6.6 °C. Attenuated total reflectance-Fourier transform
26 infrared spectroscopy (ATR-FTIR) and near-edge X-ray absorption fine structure (NEXAFS)
27 analyses revealed that Fe-bound OC was less aliphatic than non-Fe-bound OC. Fe-bound OC

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

35

36 **1 Introduction**

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

44 Currently, there is **an information** gap concerning the stability and residence time of OC,
45 contributing to the problem that the residence time of OC (ranging from months to hundreds of
46 years) is a major source of uncertainty in modeling and prediction of C cycles (Schmidt et al.,
47 2011; Riley et al., 2014). Many concepts have been proposed to account for OC stabilization and
48 therefore residence times, including molecular recalcitrance, physical occlusion, and chemical
49 protection (Sollins et al., 1996; Krull et al., 2003; Baldock et al., 2004; Mayer et al., 2004;
50 Zimmerman et al., 2004; Schmidt et al., 2011). In general, the stability of OC is regulated by
51 biogeochemical reactions occurring at the interfaces between OC, minerals, and microorganisms,
52 and further knowledge about the mechanism for OC stabilization is critical for building up
53 process-based models to simulate and predict C cycles.

54 A number of lines of evidence suggest a key importance of iron oxide minerals in the
55 stabilization of OC (Kalbitz et al., 2005; Kaiser and Guggenberger, 2007; Wagai and Mayer,
56 2007). Iron oxides have a relatively high sorption capacity for OC, with sorption coefficients for
57 OC much higher than that of other metal oxides (Kaiser and Guggenberger, 2007; Chorover and

58 Amistadi, 2001). Wagai and Mayer (2007) reported Fe-bound OC concentrations in soils up to
59 22 mg g⁻¹ soil, contributing up to 40% of total OC (TOC) for most forest soils. Similarly,
60 Lalonde et al. (2012) found that Fe-bound OC contributed 22% of TOC in sediments. Studies
61 have shown that Fe minerals protect OC from degradation and inhibit mineralization of OC
62 (Baldock and Skjemstad, 2000; Kalbitz et al., 2005). There is, however, no systematic study on
63 the occurrence of Fe-bound OC across different forests and its governing factors.

64 The overall goals of this study were to investigate the spatial variability of Fe-bound OC
65 across forest soils, **the factors that control Fe-bound OC concentrations**, and the characteristics of
66 Fe-bound OC **with respect to the** physicochemical properties of soils. In this study, we first
67 quantified the concentration of Fe-bound OC across 14 forest soils in the United States and
68 analyzed the spatial distribution and influences of ecogeographical factors. Second, we
69 investigated the impact of soil physicochemical properties on the Fe-OC associations. Third, we
70 studied molecular characteristics of Fe-bound OC **vs.** non-Fe-bound OC, including how Fe-OC
71 association influenced the chemical properties of OC and **the** stable isotope composition. Hence,
72 this study provided a systematic evaluation for the Fe-bound OC in United States forests, the
73 influences of ecological factors on the occurrence of Fe-bound OC, and the effects of association
74 with Fe on the chemical properties of OC.

75

76 **2. Methods & Materials**

77 **2.1 Chemicals and materials**

78 Reagents used for Fe reduction experiments include sodium bicarbonate (NaHCO₃:
79 Sigma-Aldrich, St. Louis, MO, USA), trisodium citrate dihydrate (Na₃C₆H₅O₇•2H₂O: Acros
80 Organics, New Jersey, USA), and sodium dithionite (Na₂S₂O₄: Alfa Aesar, Ward Hill, MA,
81 USA). All chemicals used were analytical grade.

82

83 **2.2 Soil sample collection, primary characterization and pretreatment**

84 Soil samples were collected from 14 forest sites in the United States (Obrist et al., 2011,
85 2012, 2015). The abbreviations and the basic information for the sites are summarized in Table 1.
86 More detailed information on the sites and sampling protocols can be found in previous
87 publications (Obrist et al., 2011, 2012, 2015). **Briefly, two replicate plots at each forest site were**

88 sampled. During 2007-2009, top soils (0-20 cm) from all sites were collected using clean latex
89 gloves and stainless steel sampling equipment. All the samples were immediately transferred to
90 plastic freezer bags and kept on ice before transportation to the laboratory. Soil texture was
91 analyzed by an ASTM 152-type hydrometer at the Soil Forage and Water Analysis Laboratory at
92 Oklahoma State University (Obrist et al., 2011). The soil pH was measured by mixing soil
93 particle with deionized (DI) water in a solid/solution ratio of 1:1 (Kalra, 1995). Soil samples
94 used in the experiments in this study were ground to $< 500 \mu\text{m}$ and freeze-dried after the removal
95 of roots and visible plant material and large particles ($>2 \text{ mm}$) by dry sieving.

96

97 **Table 1**

98

99 **2.3 Total C (TC), TOC and stable C isotope analyses**

100 TC, TOC and stable C isotopic compositions of soil samples were analyzed using a
101 Eurovector elemental analyzer (Eurovector SPA, Milan, Italy) interfaced to a Micromass
102 IsoPrime stable isotope ratio mass spectrometer (Micromass UK Ltd., Manchester, UK).
103 Acetanilide (71.09 % C by weight) was used as a standard compound to establish a calibration
104 curve between mass of C and the m/z 44 response from the mass spectrometer. In this study, the
105 concentration of TC and TOC were expressed as weight %. Stable C isotope analyses were
106 performed after the method of Werner et al. (1999), with results reported in the usual delta
107 notation in units of ‰ vs. Vienna Pee Dee Belemnite (VPDB). For TOC analysis, soil samples
108 were acidified with 1 M HCl with the solution/solid ratio of 1 mL solution/0.5 g soil and heated
109 at 100°C for 1 hour. The treatment was repeated three times until there was no further
110 effervescence upon acid addition, after which the samples were dried and analyzed. All analyses
111 are based on standard curves with $R^2 > 0.99$. The detection limit for C is 0.2 mg g^{-1} soil. The
112 average coefficient of variation for the analysis of C is 20.2%.

113

114 **2.4 Nitrogen (N) analysis**

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

119 reduction release treatment for each sample. All analyses are based on standard curves with
120 $R^2 > 0.99$. The detection limit for N is 0.2 mg g⁻¹ soil. The average coefficient of variation for the
121 analysis of N is 20.5%.

122

123 2.5 Analysis of Fe-bound OC

124 The concentration of Fe-bound OC was quantified by an established Fe reduction release
125 method, commonly known as DCB extraction involving sodium dithionite, citrate and
126 bicarbonate (Mehra and Jackson, 1960; Wagai and Mayer, 2007; Lalonde et al., 2012). The DCB
127 extraction is assumed to extract most free Fe oxides (i.e. goethite, hematite, ferrihydrite and
128 others) existing in soils, but should not extract structural Fe in clay minerals (Mehra and Jackson,
129 1960; Wagai and Mayer et al., 2007; Lalonde et al., 2012). In this study, we followed the specific
130 protocol detailed in Lalonde et al. (2012). An aliquot (0.25 g) of soil was mixed with 15 mL of
131 buffer solution at pH 7 (containing 0.11 M bicarbonate and 0.27 M trisodium citrate), and then
132 heated to 80°C in a water bath. The reducing agent sodium dithionite was added to the samples
133 with final concentration of 0.1 M, and maintained at 80°C for 15 min. The samples were then
134 centrifuged at 10,000 rpm for 10 min, the supernatant was removed, and the residual particles
135 were rinsed using 5 mL of DI water. The rinse/centrifuge process was performed three times.
136 The residual particles were freeze-dried and analyzed for TC and TOC concentrations and $\delta^{13}\text{C}$
137 composition. The mass of residual particles was used to calculate the OC concentration
138 associated with non-Fe minerals.

139 The background release of OC during the heating process was measured following the
140 method in Lalonde et al. (2012), where sodium citrate and dithionite were replaced by sodium
141 chloride with the same ionic strength. An aliquot (0.25 g) of dry soil was mixed with 15 mL of
142 1.6 M NaCl and 0.11 M NaHCO₃, and heated to 80°C. Then 0.22 g of NaCl was added, and the
143 solution was maintained at 80°C for 15 min. The samples were then centrifuged at 10,000 rpm
144 and rinsed three times, and freeze-dried before analysis. The mass of residual particles was used
145 to calculate the concentration of OC released by heating to 80°C. In preliminary experiments, we
146 found that the solution pH increased rapidly during the heating-extraction process with
147 bicarbonate and sodium chloride only, and the increased pH values facilitated the release of
148 additional OC. Hence, we used a lower initial pH of 6 to compensate for the shift to higher pH
149 during heating. To validate the measurement for the concentration of OC released during heating,

150 we also tested the release of OC using a phosphate buffer (same ionic strength) in lieu of the
151 bicarbonate buffer, which can maintain a pH of 7 during heating. Our results showed that the
152 concentration of OC released was similar for both the bicarbonate and phosphate buffer
153 extraction reactions (Supplementary Material, Fig. S1).

154

155 **2.6 Quantification of reactive Fe**

156 The concentration of reactive Fe in soils was determined by analyzing the Fe released
157 during the DCB reduction process. After the reduction treatment, the supernatant of each sample
158 was filtered using a 0.2 μm syringe filter (cellulose acetate), and analyzed for Fe concentration
159 by inductively coupled plasma - atomic emission spectroscopy (Varian-Vista AX CCD, Palo
160 Alto, CA, USA) at an optical absorption wavelength of 259.9 nm. All analyses are based on
161 standard curves with $R^2 > 0.99$. The detection limit for Fe is 0.04 mg g^{-1} soil. The average
162 coefficient of variation for the analysis of Fe is 25.8%.

163

164 **2.7 Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR)**

165 ATR-FTIR analysis to characterize the molecular composition of OC was performed for
166 original soil samples and residual soils after DCB extraction using a Thermo Scientific Nicolet
167 6700 FTIR (Waltham, MA). Dry soil samples were placed directly on the crystal and forced to
168 contact well with the crystal. Spectra were acquired at the resolution of 4 cm^{-1} based on 100
169 scans. Data collection and baseline correction were accomplished using OMNIC software
170 version 8.3.103.

171

172 **2.8 Near-edge X-ray absorption fine structure (NEXAFS) analysis**

173 For further characterization of chemical structure of OM, carbon (1s) K-edge NEXAFS
174 analyses were performed for select soil samples, i.e. for soils with the highest and lowest values
175 of the fraction of Fe-bound OC to TOC. The soil particles were suspended in DI water and
176 deposited on an Au-coated silicon wafer attached to a Cu sample holder. Before analysis,
177 samples were dried in a vacuum desiccator. The X-ray-based experiments were performed on the
178 Spherical Grating Monochromator (SGM) beamline at the Canadian Light Source (Saskatoon,
179 Canada) (Regier et al., 2007). The energy scale was calibrated using citric acid (absorption at
180 288.6 eV). Major technical parameters and set-up for the beamline include: X-ray energy ranges

181 250-2000 eV; 45 mm planer undulator; 1000 $\mu\text{m}\times 100\ \mu\text{m}$ spot size; silicon drift detectors (SDD);
182 a titanium filter before the sample; entrance and exit slit gaps of 249.9 μm and 25 μm (Gillespie
183 et al., 2015). Carbon 1s spectra were acquired by slew scans from 270 to 320 eV at 20 s dwell
184 time and 20 scans per sample on a new spot. For data normalization, I_0 was collected by
185 measuring the scatter of the incident beam from a freshly Au-coated Si wafer using SDD.
186 Before the I_0 normalization, the pre-edge baseline was adjusted to near zero to remove the scatter
187 in the sample data (Gillespie et al., 2015).

188 3. Results and Discussion

189 3.1 Concentration of Fe-bound OC

190 This study covered five major forest types in North America, including Spruce-Fir, Pine,
191 Oak, Chaparral, and Maple-beech-birch forests distributed between 29° and 47° N. For the 14
192 forest soils, TC concentrations ranged between 1.5 ± 0.1 and $8.3\pm 2.1\%$ (all percentages given are
193 weight-based), and TOC concentrations ranged between 1.3 ± 0.3 and $6.2\pm 2.9\%$, which are
194 comparable to values previously reported for North American forest soils (Wagai and Mayer,
195 2007; Wilson et al., 2013). Bicarbonate extraction-calibrated Fe-bound OC concentrations
196 ranged from 0.3 to 1.9%, with the fraction of Fe-bound OC to TOC ($f_{\text{Fe-OC}}$) averaging $37.8\pm 20.0\%$
197 (Fig. 1, Supplementary Material, Table S1). Forest HL (Maine) had the highest $f_{\text{Fe-OC}}$ of 57.8%,
198 while forests GS (Florida) and OR (Tennessee) had $f_{\text{Fe-OC}}$ values below detection limits (i.e.,
199 below 0.6%). Based on an estimate that 1502 Pg ($\text{Pg}=1\times 10^{15}\ \text{g}$) of TOC is stored in terrestrial
200 soils (Scharlemann, et al., 2014), scaling up these results to a global estimate would yield
201 538.5 ± 271.5 Pg of Fe-bound OC residing in terrestrial soils.

202

203 Fig. 1

204

205 3.2 Fe-OC association

206 The values of $f_{\text{Fe-OC}}$ were influenced not only by the concentration of reactive Fe, but also
207 by the type of association between Fe and OC. In this study, the concentration of reactive Fe in
208 forest soils ranged from $0.1\ \text{mg g}^{-1}$ to $19.3\ \text{mg g}^{-1}$, which is low compared to values of reactive
209 Fe of up to $180\ \text{mg g}^{-1}$ reported previously (Wagai and Mayer, 2007; Wagai et al., 2013) (Fig.
210 2A). A Mollisol in forest site MS (California) had the highest concentration of reactive Fe, while

211 a Spodosol in forest site GS (Florida) had the lowest reactive Fe concentration. There was no
212 significant correlation between $f_{\text{Fe-OC}}$ and the concentration of reactive Fe (Pearson Correlation
213 Coefficient $r=-0.418$, $p=0.137$, Fig. 2B). This suggests that the proportion of Fe-bound OC is not
214 strongly controlled by the reactive Fe concentration.

215 The OC:Fe molar ratio ranged from 0.56 to 17.7 for all 14 soils, with a value between 1
216 and 10 for 10 soils (Fig. 2A). Previous studies have suggested that the OC:Fe molar ratio can be
217 used as an indicator for the type of association between Fe oxides and OC, with lower values
218 indicating sorptive interactions while higher values indicate incorporation of OC within Fe
219 oxides (Wagai et al., 2007; Guggenberger and Kaiser, 2003). The highest sorption capacity
220 measured for OC onto Fe oxide corresponds to an OC:Fe molar ratio = 1.0 (Kaiser and
221 Guggenberger, 2006), but by incorporation and co-precipitation of Fe oxide OC:Fe molar ratio
222 can reach much higher values (Guggenberger and Kaiser, 2003). With OC:Fe molar ratios
223 generally between 1-10 for about two thirds of the forest soils in this study, we propose that
224 incorporation of OC into Fe oxides plays a major role in the accumulation of Fe-bound OC
225 exceeding sorption by at least a factor of 1 to almost 20 (Wagai and Mayer, 2007; Lalonde, 2012).
226 However, for the HT (Michigan), HL (Maine) and TKF (California) forest soils, the OC:Fe
227 molar ratios were even higher than 10 with a maximum value of 17.8 (Fig. 2A), implying that
228 incorporation of OC into Fe oxides dominated at these sites. Similar to $f_{\text{Fe-OC}}$, OC:Fe ratios were
229 not related to the concentration of reactive Fe and showed large variation for soils with similar
230 concentration of total reactive Fe (Fig. 2B). This further indicates that the type of interactions
231 between OC and Fe was not governed by the amount of Fe. The OC:Fe ratio is potentially
232 regulated by the mineral phases of Fe, as poorly-crystalline Fe oxides have a higher capacity to
233 bind with OC than crystalline Fe minerals (Eusterhues et al., 2014). When sorption dominates
234 the interactions between OC and Fe, OC:Fe can also be influenced greatly by the particle size
235 and surface area of Fe oxides (Gu et al., 1995). Further investigations are needed to determine
236 the factors that control the OC:Fe ratio, and also $f_{\text{Fe-OC}}$ values for soils. Nevertheless, the lack of
237 (or poor) relationship shown here between the concentration of Fe-bound OC and Fe
238 concentrations demonstrates the limitations associated with predicting and modeling the behavior
239 of C in forest soils based on the Fe concentrations in soils alone.

240

241 **Fig. 2.**

242

243 **3.3 Spatial variance and ecogeographical factors**

244 We analyzed the influences of ecogeographical factors on the occurrence of Fe-bound OC in
245 forest soils (Fig. 3). There was a significant correlation between the TOC concentration and
246 latitude (Pearson correlation coefficient $p=0.619$, $r=0.018$), a pattern commonly observed due to
247 lower microbial activity and turnover rates of C at higher, colder latitudes (Davidson and
248 Janssens, 2006). The concentration of reactive Fe, if excluding soil MS in California, was also
249 significantly related to latitude ($p=0.824$, $r=0.001$). Both concentrations of Fe-bound OC and $f_{\text{Fe-OC}}$
250 were also correlated with latitude ($p=0.523$, $r=0.053$; $p=0.525$, $r=0.054$). Among our samples,
251 the soil in forest HL in Maine, one of the three northern-most site with latitude of 45° , had the
252 highest $f_{\text{Fe-OC}}$ of 57.8%. In forest GS in Florida with lowest latitude of 29.7° , the $f_{\text{Fe-OC}}$ was below
253 detection limits, possibly due to the low concentration of reactive Fe (0.08 mg g^{-1}). Hence,
254 increase in latitude both increased concentrations of TOC in soil as well concentrations of Fe-
255 bound OC, suggesting increased interactions between Fe oxide and OC at higher latitudes. There
256 were no clear trends in TOC or Fe-OC interactions with longitude. For elevation, we separated
257 two groups of samples, with one group located below 1000 m (asl) and the other group above
258 (mainly around 2000 and 4000 asl). Concentrations of TOC and Fe-bound OC, however, were
259 not significantly different between the two groups. There were no clear trends with precipitation
260 either, although others have reported positive relationships between mean annual precipitation
261 and soil TOC concentration at a global scale (Amundson, 2001). The concentration of Fe-bound
262 OC and $f_{\text{Fe-OC}}$ reached peak value with mean annual temperatures at 6.6°C , with lower values
263 both at higher and lower temperatures. Temperature dependence of Fe-bound OC can be
264 regulated by effects of temperature on the mineral phase of Fe oxides and OC dynamics. Given
265 that ferrihydrite can incorporate more OC than other crystalline Fe oxides, an increase in
266 temperature favors the transformation of ferrihydrite to other crystalline iron oxides
267 (Gnanaprakash et al., 2007; Zhao et al., 1994). However, an increase in temperature can also
268 accelerate weathering of other minerals, and increased release of silicon can slow the
269 transformation of ferrihydrite (Cornell et al., 1987; White and Blum, 1995). However, there is
270 also evidence that temperature can affect the chemical composition of soil OC substantially
271 (Conant et al., 2011). For example, increased temperature decreased the content of oxidized

272 functional groups, such as saccharides, which would consequently inhibit the interactions
273 between OC and Fe oxides (Amelung et al., 1997). The overall pattern can result from combined
274 effects of temperature on Fe mineral phase and OC transformation. Further investigations are
275 required to elucidate the mechanism more accurately. Finally, the study covered 7 major soil
276 orders, i.e. Alfisols (sample number n=3), Spodosols (n=4), Mollisols (n=1), Inceptisols (n=2),
277 Entisols (n=2), Gelisols (n=1), and Ultisols (n=1). Although there are limited replications in
278 many of these soil orders, the highest concentration of Fe-bound OC were observed in Spodosols.
279 Regarding $f_{\text{Fe-OC}}$, the highest values were also found in Spodosols, possibly indicating a
280 particular importance of Fe-bound OC in this soil type which occupies 3.5% of US land areas
281 and 4% of global ice-free land (Soil Survey Staff, 1999). However, due to the limited number of
282 samples for each soil order, these findings warrant further confirmation.

283

284 **Fig. 3**

285

286 **3.4 Impact of soil physicochemical properties on Fe-OC association**

287 Soil texture can potentially influence the accumulation of Fe-bound OC. Figure 4
288 demonstrates that the fraction of non-calibrated Fe-bound OC showed a significant positive
289 correlation with the fraction of sand ($r=0.72$, $p<0.001$), and negative correlations with the
290 fraction of silt ($r=-0.697$, $p<0.001$) and clay ($r=-0.616$, $p<0.001$). There were similar positive
291 correlations between labile OC, and the fraction of sand ($r=0.72$, $p<0.001$), silt ($r=0.72$, $p<0.001$)
292 and clay ($r=0.72$, $p<0.001$). However, the calibrated Fe-bound OC had no significant correlation
293 with any of the texture fractions. These correlations indicate that the labile OC was mainly
294 associated with the sand component of forest soils, but that the soil texture did not affect the Fe-
295 bound OC. There is debate on the relative roles of sand, clay and silt in the stabilization of OC in
296 soil (Percival et al., 2000; Six et al., 2002; Eusterhues et al., 2005; Vogel et al., 2014).
297 Eusterhues et al. (2005) found a relationship between the resistance of organic matter to
298 oxidative degradation and the clay concentration in soils, suggesting the importance of clay
299 minerals in the stabilization and accumulation of soil OC. Reduced chemical potential of soil
300 organic matter in small pores of clay-rich soils also limits microbial degradation and enhance its
301 stabilization (Riedel and Weber, 2016). In contrast, Percival et al. (2000) found that the clay
302 mineral fraction explained little of the variation in the accumulation of OC across a range of soil

303 types in New Zealand. Vogel et al. (2014) found that less than 20% of clay mineral surfaces were
304 covered by the sorption of OC, indicating that a limited proportion of clay mineral surface
305 contributed towards the stabilization of OC. Our results suggest that the Fe oxide-mediated
306 stabilization of OC was not related to the size/aggregation-based process, although the labile
307 carbon concentrations increased with the fraction of sand in the soils.

308

309 **Fig. 4**

310

311 The Fe-OC association can also be influenced by the soil pH, which affects the mineral
312 phases of Fe oxides and their surface charge, and their interactions with OC. For our soil samples,
313 the soil pH ranged from 4.1 to 6.3, similar to measurements by Wagai and Mayer (2007) for
314 North America soils. There was no significant correlation between the $f_{\text{Fe-OC}}$ and soil pH, e.g. the
315 HL (Maine) soil with pH of 4.4 had the highest $f_{\text{Fe-OC}}$ of 57.8%, while the TS(II) (Washington)
316 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,
317 $f_{\text{Fe-OC}}$ did not change correspondingly. Contrastingly, values of OC:Fe molar ratios were
318 significantly influenced by the soil pH; **except** for one outlier sample of TS(II) (Washington)
319 soil, there was a significant negative correlation between the OC:Fe molar ratio and soil pH ($r=-$
320 0.477 , $p=0.09$) (Supplementary Material, Fig. S2). This may be due to the lower pH values
321 favoring the complexation and precipitation of Fe with OC, while higher pH favors sorptive
322 interactions between Fe minerals and OC (Tipping et al., 2002). If comparing samples with
323 similar pH, the soils with higher TOC had higher OC:Fe molar ratios, e.g. the GS soil (TOC =
324 1.1%) with pH of 4.7 had an OC:Fe molar ratio = 8.5, while the HT (Michigan) soil (TOC =
325 3.0%) with similar pH of 4.7 had an OC:Fe molar ratio = 17.1. **This was consistent with**
326 **Schwertmann et al. (1986), who found that the major form of Fe would change from FeO_x to**
327 **complexes with OC when there is higher OC supply.**

328

329 **3.5 Molecular characteristics of Fe-bound OC**

330 The chemical composition of Fe-bound OC can be substantially different from non-Fe-
331 bound OC (Adhikari and Yang, 2015) with broad implications on the C biogeochemical cycles,
332 although such differences so far have received limited attention. We analyzed the difference in
333 chemical composition of Fe-bound OC compared to non-Fe-bound OC using ATR-FTIR

334 analysis (Fig. 5). Overall, there were limited fingerprint peaks for OC, because of the low
335 concentration of TOC and technical challenge for analyzing whole soil particles with FTIR
336 (Calderon et al., 2011; Simonetti et al., 2012). Reeves (2012) demonstrated that FTIR analysis of
337 mineral soils in the ranges of 1600-1750 and 2800-3000 cm^{-1} only can be used to study OC.
338 Peaks in the range of 500-1200 cm^{-1} indicate the presence of clay or other Fe/Al minerals (Fig. 5)
339 (Madejova, 2003; Harsh et al., 2002; Parikh et al., 2014), such as kaolinite or montmorillonite at
340 850-1200 cm^{-1} (Madejova, 2003). Absorption at 850-1200 cm^{-1} can also be due to the presence
341 of polysaccharides, but definitive identification of polysaccharides is not possible in the presence
342 of minerals (Senesi et al., 2003; Tandy et al., 2010). The spectra in the range of 1600-1750 cm^{-1}
343 normally contain fingerprint peaks for functional groups of amides, carboxylates and aromatics
344 (Parikh et al., 2014), but we did not detect any significant peaks in this range. In the range of
345 2800-3000 cm^{-1} , there were no significant peaks for the original soil samples, but after Fe
346 extraction we detected significant peaks at 2850 and 2930 cm^{-1} , which are characteristic for the
347 presence of aliphatic carbon. The substantial differences in spectra before and after Fe extraction
348 indicate that aliphatic OC was enriched in the residual soils after extraction. Other functional
349 groups, such as aromatic carbon and hydrophilic functional groups, were more strongly
350 associated with Fe minerals and removed during the Fe extraction, as hydrophilic functional
351 groups can form inner-sphere coordination complexation with iron oxides, and aromatic carbon
352 has electron donor-acceptor interactions with iron oxides (Gu et al., 1995; Axe and Persson,
353 2001). This result was consistent with a previous study using ultra-high resolution mass
354 spectrometry, showing the release of more aromatic carbon during the reductive dissolution of Fe
355 oxides (Riedel et al., 2014). Analysis for the chemical nature of Fe-bound OC can be influenced
356 by the potential reaction of natural organic matter with dithionite, which was not noticed in
357 previous studies (Lalonde et al., 2012; Wagai and Mayer, 2007). The most likely reaction
358 between dithionite and organic matter is the reduction of oxidized organic functional groups. Our
359 recent study showed that dithionite could reduce quinone groups in natural organic matter
360 (Adhikari et al., 2016). Most likely, other major functional groups, such as carboxylic and
361 carbonyl functional groups, cannot be reduced by dithionite based on their reduction potentials
362 (Bar-Even et al., 2012; Mayhew et al., 1978). Further investigations are needed to elaborate the
363 detailed influences of dithionite reduction on the molecular properties of organic matter.

364

365 **Fig. 5**

366

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

382

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

388
$$\delta^{13}\text{C}_{\text{TOC}} \times \text{TOC} = \delta^{13}\text{C}_{\text{labile}} \times \text{OC}_{\text{labile}} + \delta^{13}\text{C}'_{\text{Fe-OC}} \times \text{OC}'_{\text{Fe}} + \delta^{13}\text{C}_{\text{non-Fe-OC}} \times \text{OC}_{\text{non-Fe}} \quad (1)$$

389 where TOC is the concentration of total organic carbon, $\text{OC}_{\text{labile}}$ is the concentration of labile OC
390 (extractable by bicarbonate buffer), $\text{OC}_{\text{non-Fe}}$ is the concentration of non-Fe-bound OC (residual
391 OC after Fe extraction), and OC'_{Fe} is the concentration of Fe-bound OC (excluded the labile OC);
392 $\delta^{13}\text{C}_{\text{TOC}}$ 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,
393 $\delta^{13}\text{C}_{\text{non-Fe-OC}}$ is $\delta^{13}\text{C}$ for non-Fe-bound OC. However, it is difficult to directly resolve the $\delta^{13}\text{C}_{\text{labile}}$
394 and $\delta^{13}\text{C}'_{\text{Fe-OC}}$ using this equation. We simplified it to equation (2):

$$\delta^{13}\text{C}_{\text{Fe-OC}} = \frac{(\delta^{13}\text{C}_{\text{TOC}} \times \text{TOC} - \delta^{13}\text{C}_{\text{non-Fe-OC}} \times \text{OC}_{\text{non-Fe}})}{\text{OC}_{\text{Fe}}} \quad (2)$$

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 OC_{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}C ($1.5 \pm 1.2\text{‰}$ 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):

$$\delta^{13}\text{C}_{\text{labile}} = \frac{(\delta^{13}\text{C}_{\text{TOC}} \times \text{TOC} - \delta^{13}\text{C}_{\text{non-labile}} \times \text{OC}_{\text{non-labile}})}{\text{OC}_{\text{labile}}} \quad (3)$$

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}C compared to the non-Fe-bound OC in forest soils, which is consistent with results for sediments, where Fe-bound OC was $1.7 \pm 2.8\text{‰}$ 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 $\Delta^{13}\text{C}_{\text{FeOC-nonFeOC}}$ ($= \delta^{13}\text{C}_{\text{Fe-OC}} - \delta^{13}\text{C}_{\text{non-Fe-OC}}$) (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}C was to some degree

425 related to the OC:Fe ratio. As discussed previously (section 3.2), lower OC:Fe ratios indicate an
426 increased contribution from sorptive interactions of OC with Fe minerals as compared to
427 incorporation of OC within iron oxides and OC, and these sorptive interactions between oxygen-
428 and nitrogen-rich organic compounds and Fe oxide results in the enrichment of ^{13}C of Fe-bound
429 OC vs. non-Fe-bound OC. Previous studies have attributed the stability of relatively labile and
430 reactive compounds, such as amino acids and sugars, to their interactions with minerals (Schmidt
431 et al., 2011), and our results demonstrated the importance of sorption to Fe minerals in increasing
432 the stability of relatively reactive labile compounds.

433

434 Fig. 6

435

436 Nitrogen (N)-containing functional groups are potentially important for the association
437 between OC and Fe oxides, although the concentrations of N are much lower than C (Yang et al.,
438 2012; Barber et al., 2014). The bulk soil contained 0.05-0.45 % N, while the non-Fe-bound
439 component (i.e. the residual solid after DCB extraction) contained 0.06-0.32 % N.
440 Concentrations of Fe-bound N, calculated by difference, ranged up to 0.13 %. However, it is
441 important to note that this number is based without a calibration for labile N that may be
442 removed by the dithionite-free DCB extraction (data not available). There were significant
443 correlations between C and N concentrations for both bulk soils ($r=0.847$, $p<0.001$:
444 Supplementary Material, Fig. S4) and the non-Fe-bound residual components ($r=0.858$, $p<0.001$:
445 Supplementary Material, Fig. S4), with molar C/N ratios of 14.2 ± 2.6 and 13.7 ± 2.3 for bulk and
446 non-Fe-bound OC, respectively. These C/N values are essentially identical to a previously
447 observed molar C/N ratio = 14.3 for a large set of world-wide soils samples (Cleveland et al.,
448 2007), and a molar C/N ratio = 14.4 for OC-rich samples in China (Tian et al., 2010). This result
449 suggests that C/N ratios for Fe-bound OC did not differ from that of non-Fe-bound OC,
450 assuming that the labile carbon did not have a substantially different C/N ratio. Therefore, in
451 contrast to the ^{13}C enrichment observed for Fe-bound OC, the interactions with Fe minerals did
452 not affect the C/N ratio substantially.

453

454 4. Conclusion

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

467

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475

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673 **Figure Captions**

674 **Figure 1.** Concentrations of total carbon (TC), total organic carbon (TOC) and Fe-bound OC in
675 14 forest soils across the United States. Duplicate measurements were conducted for each of two
676 plots in every forest site. Error bars represent standard deviation of measurements of four
677 replicates for each forest site.

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

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

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

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

690 **Figure 6. A.** $\delta^{13}\text{C}$ of total organic carbon and non-iron bound organic carbon for 14 U.S. forest
691 sites. **B.** Correlation between $\Delta^{13}\text{C}_{\text{FeOC-nonFeOC}}$ and molar ratio of OC:Fe.

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

| Forest ID | Abbr. | Location | Soil Order (US) | Soil Class ^a (FAO) | Climate Zone | Precip. ^b (mm y ⁻¹) | Temp ^c (°C) | LAT(°) ^d | LONG (°) ^e | Elevation (m asl) |
|---------------------------|-------|-------------------------|-----------------|-------------------------------|-----------------------|--|------------------------|---------------------|-----------------------|-------------------|
| Ashland | AL | Ashland, Missouri | Alfisols | Luvisols & Greyzems | Humid Continental | 1023 | 13.9 | 38.73 | -92.20 | 210 |
| Bartlett | BL | Bartlett, New Hampshire | Spodosols | Podzols & Lithosols | Humid Continental | 1300 | 4.5 | 44.0 | -71.29 | 272 |
| Marysville | MS | Marysville, California | Mollisols | Luvisols | Mediterranean climate | 775 | 16.9 | 39.25 | -121.28 | 386 |
| Gainesville | GS | Gainesville, Florida | Spodosols | Podzols | Humid Subtropical | 1228 | 21.7 | 29.74 | -82.22 | 50 |
| Oak Ridge | OR | Oak Ridge, Tennessee | Ultisols | Acrisols | Humid Subtropical | 1350 | 14.5 | 35.97 | -84.28 | |
| Little Valley (post-fire) | LVF | Little Valley, Nevada | Entisols | Arenosols | Highland Climate | 551 | 5.0 | 39.12 | -119.93 | 2010 |
| Little Valley | LV | Little Valley, Nevada | Entisols | Arenosols | Highland Climate | 550 | 5.0 | 39.12 | -119.93 | 2011 |
| Truckee (post-fire) | TKF | Truckee, California | Alfisols | Luvisols | Highland Climate | 569 | 6.0 | 39.37 | -120.1 | 1768 |
| Truckee | TK | Truckee, California | Alfisols | Luvisols | Highland Climate | 568 | 5.9 | 39.37 | -120.1 | 1767 |
| Niwot Ridge | NR | Niwot Ridge, Colorado | Alfisols | Cambisols | Highland Climate | 800 | 1.3 | 40.03 | -105.55 | 3050 |
| Hart | HT | Hart, Michigan | Spodosols | Podzols | Humid Continental | 812 | 7.6 | 43.67 | -86.15 | 210 |
| Howland | HL | Howland, Maine | Spodosols | Luvisols | Humid Continental | 1040 | 6.7 | 45.20 | -68.74 | 60 |
| Thompson I | TSI | Ravensdale, Washington | Inceptisols | Cambisols | Highland Climate | 1141 | 9.8 | 47.38 | -121.93 | 221 |
| Thompson II | TSII | Ravensdale, Washington | Inceptisols | Cambisols | Highland Climate | 1140 | 9.8 | 47.38 | -121.93 | 220 |

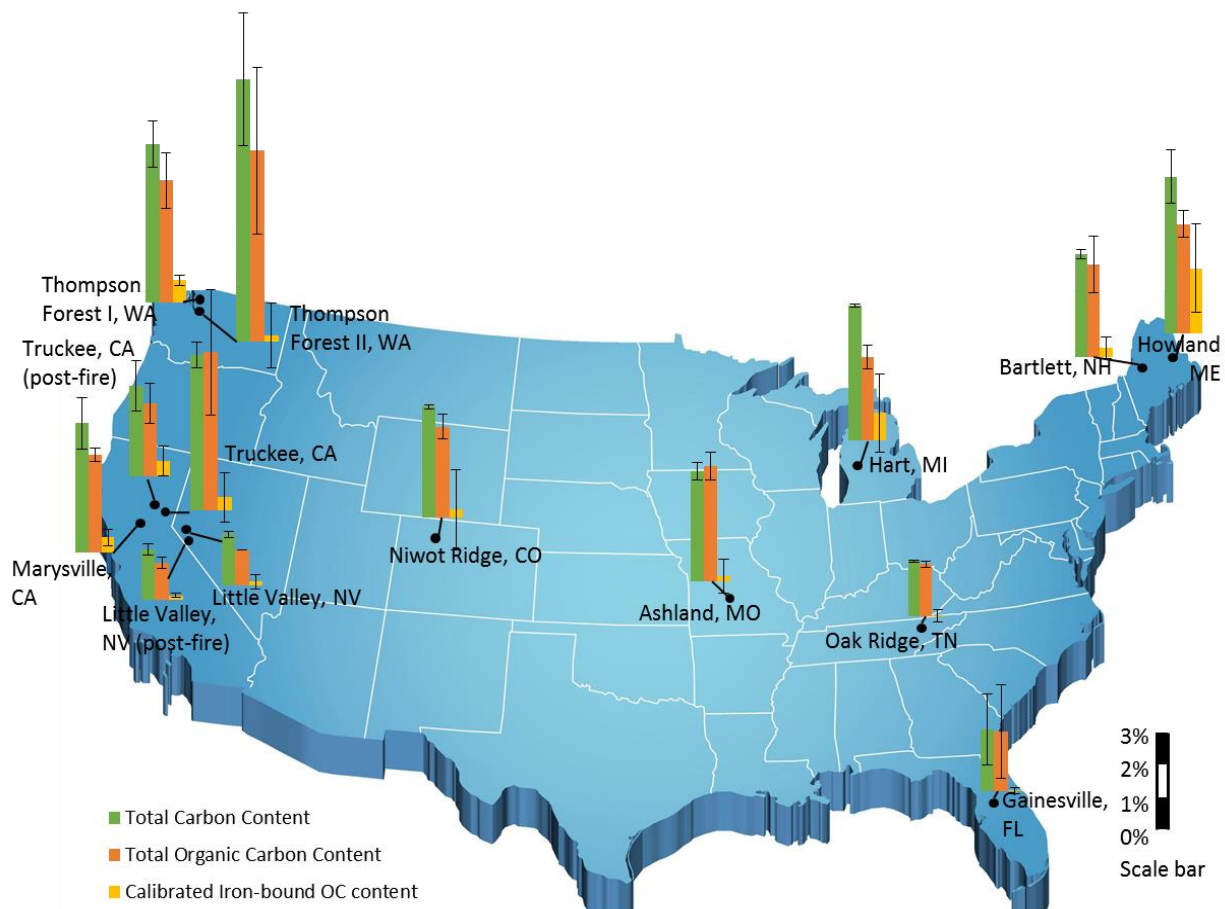
a: Food and Agriculture Organization; b: annual precipitation; c: annual mean temperature; d latitude; e: longitude.

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706 **Fig. 1**

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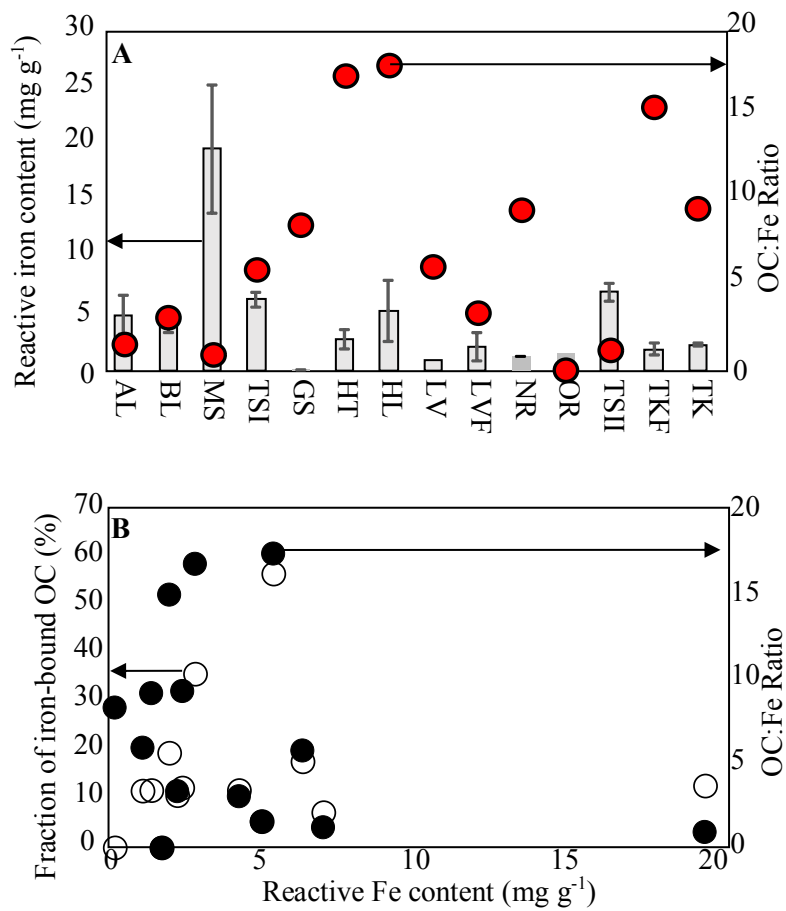
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719 **Fig. 2**

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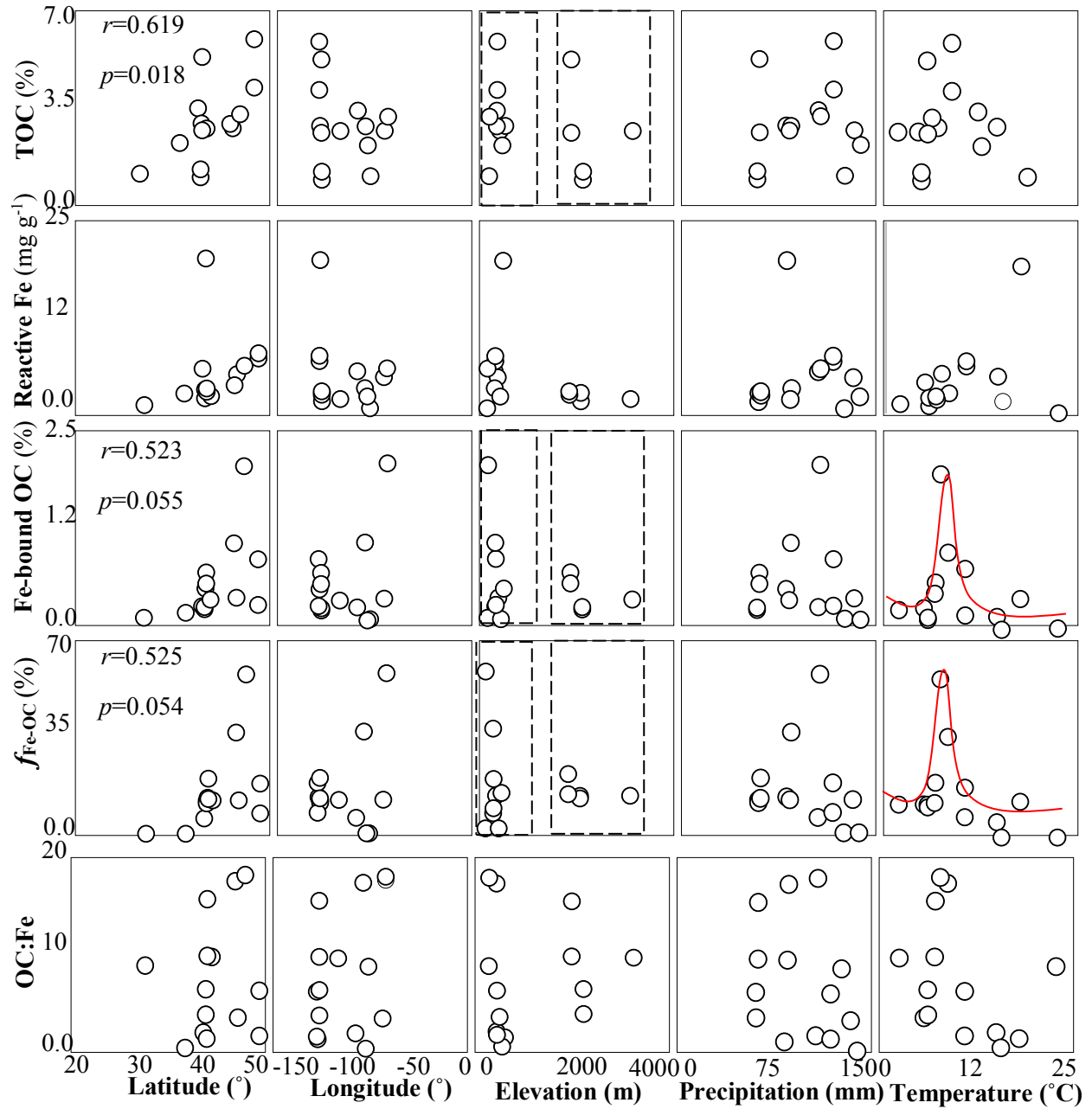
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733 **Fig. 3**

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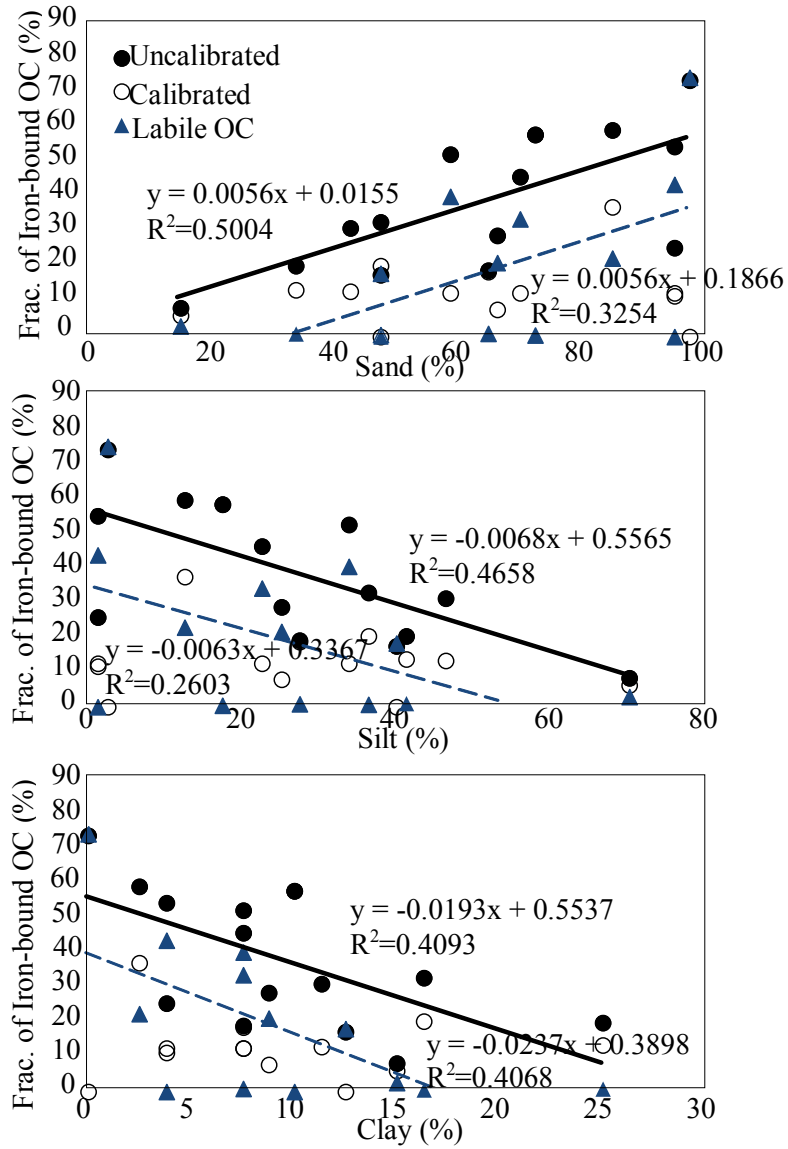
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741 **Fig. 4**

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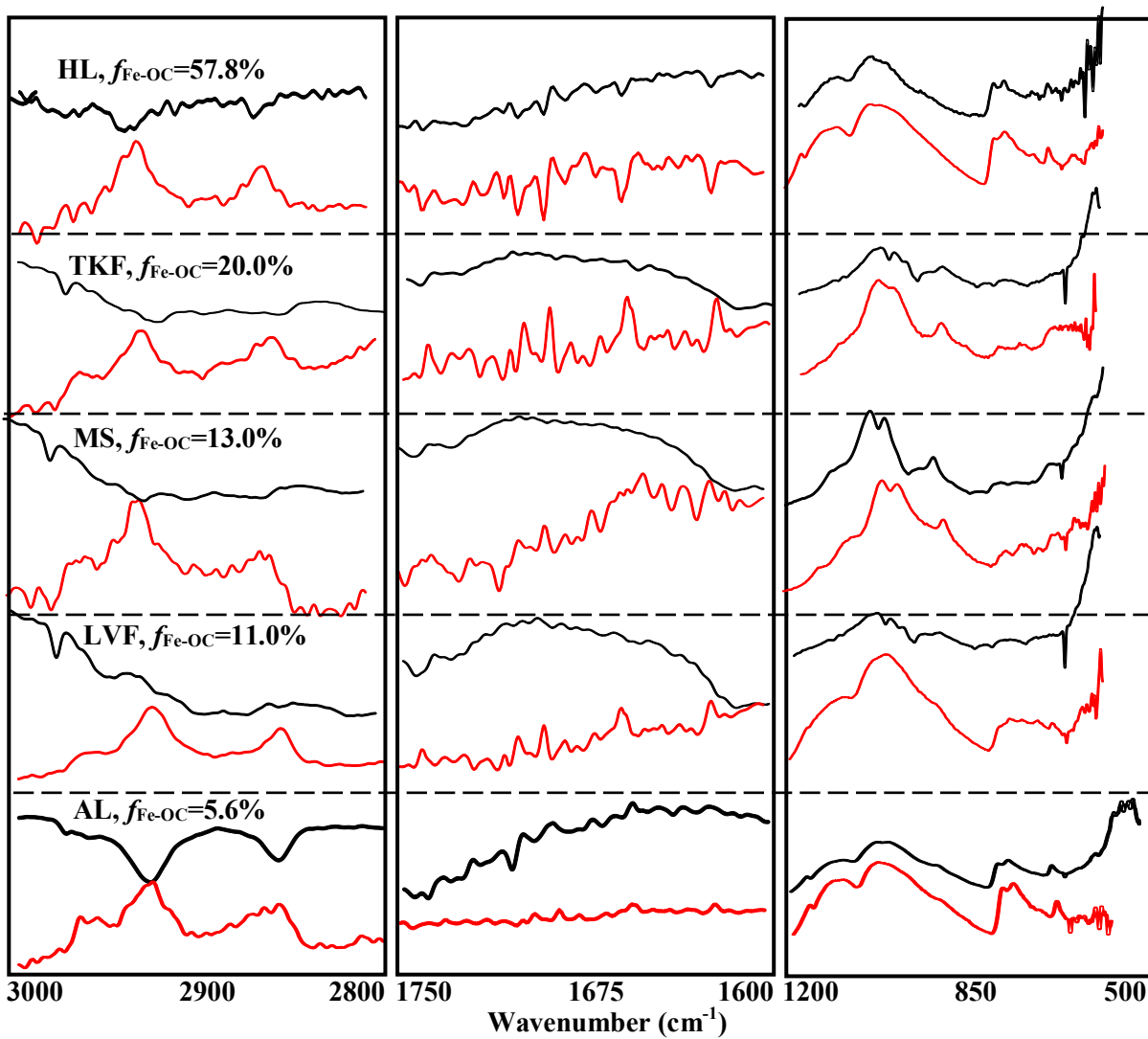
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752 Fig. 5

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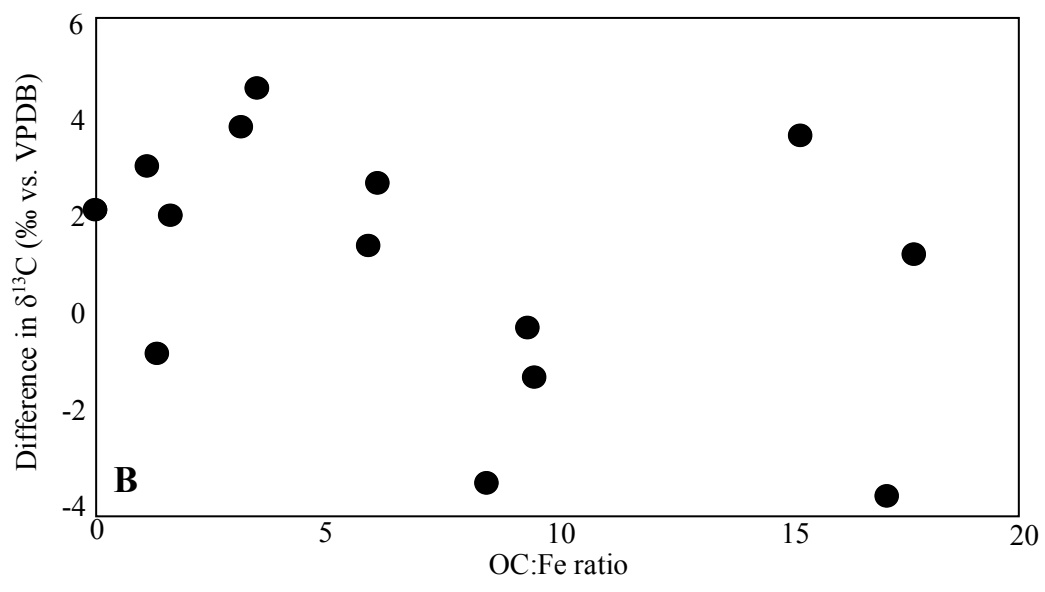
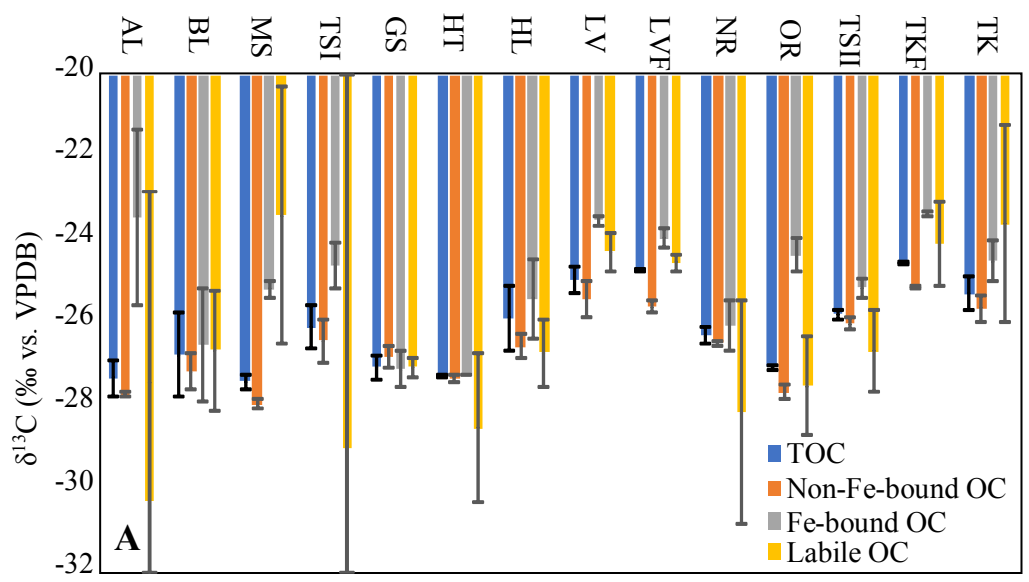
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Fig. 6