**Asynchronous reductive release of iron and organic carbon from hematite-humic acid
complexes**

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ABSTRACT

Solid-phase iron (Fe) plays an important role in the accumulation and stabilization of soil organic matter (SOM). However, ferric minerals are subject to redox reactions, which can compromise the stability of Fe-bound SOM. To date, there is limited information available concerning the fate of Fe-bound SOM during redox reactions at Fe mineral surfaces. In this study, we investigated the release kinetics of hematite-bound organic carbon (OC) during the abiotic reduction of hematite-humic acid (HA) complexes by dithionite, to elucidate important processes governing the stability and fate of organic matter during the redox processes. Our results indicate that the reductive release of Fe obeyed first-order kinetics with release rate constants of 6.67-13.0 $\times 10^{-3}$ min⁻¹. The Fe-bound OC was released rapidly during the initial stage with release rate constants of 0.011-1.49 min⁻¹, and then became stable with residual fractions of 4.6-58.2% between 120 and 240 min. The release rate of aromatic OC was much faster than for the non-aromatic fraction of HA, and 90% of aromatic OC was released within the first hour for most samples. Our findings show that in the reductive reaction the mobilization of Fe-bound OC was asynchronous with the reduction of Fe, and aromatic OC was released more readily than other components of SOM. This study highlights the importance of evaluating the release of SOM bound with Fe during the redox reactions, especially the influence of the physicochemical properties of SOM.

Keywords: Soil organic matter; Iron oxide; Reduction; Release; Stability

1. Introduction

Soil organic matter (SOM) contains 1500 gigatons (Gt) of carbon, approximately double the amount of carbon present in the atmosphere and triple that in terrestrial vegetation (Amundson, 2001; Houghton, 2007; Lal, 2004). A significant amount of carbon (~3.5 Gt per year) is released from soil to the atmosphere as a result of SOM decomposition, contributing to an increase in the concentration of greenhouse gases (Houghton, 2007). Conversely, if effective soil management practices are implemented, SOM can act as a sink for atmospheric carbon, and help mitigate the harmful effects of global warming (Houghton, 2007; Kaiser and Guggenberger, 2003). The soil carbon reservoir can be beneficial as a carbon sink only if SOM is stabilized and stored for relatively long periods. Understanding the stabilization of SOM is important for not only evaluating and predicting the biogeochemical cycles of carbon, but also for effectively managing soil ecosystems under global climate change.

Iron (Fe) oxides have been suggested as important mineral phases for stabilizing SOM, as SOM can be sorbed onto Fe oxides with high sorption affinity (Kaiser and Guggenberger, 2000; Kaiser and Zech, 2000; Lalonde et al., 2012). The sorption capacity for organic matter onto Fe oxide is 110-140 mg C/g, approximately ten times the value of aluminum oxides (Kaiser and Guggenberger, 2007; Tipping, 1981). As a result, over 20% of SOM in soil can be bound to Fe oxides (Wagai and Mayer, 2007). The sorptive association with minerals can protect SOM from degradation by physicochemical sequestration, thereby enhancing SOM stability (Torn et al., 1997; Zimmerman et al., 2002). Hence, understanding the fate of Fe-bound SOM is critical for evaluating the stability of SOM.

Fe(III) oxides are subject to redox reactions, which can break down Fe(III) oxide-SOM complexes and compromise the stability of Fe-bound SOM. Redox reactions of Fe(III)/Fe(II) can

be mediated by various abiotic and biotic agents in the soil environment, under the anaerobic-aerobic transitions which occur in natural soils (Chacon et al., 2006; Lipson et al., 2010; Rivkina et al., 1998). Many metal-respiring bacteria derive energy from the reduction of Fe(III) for cell growth and microbial activities (Brock and Gustafson, 1976; Lovley, 1997; Lovley and Phillips, 1986; Lovley and Phillips, 1988). Previous geobiological studies have documented the importance of SOM in Fe redox reactions, where it may act as an electron shuttle, electron donor or electron acceptor (Kappler et al., 2004; Lovley, 1997; Lovley et al., 1996; Lovley and Phillips, 1986; Roden et al., 2010; Scott et al., 1998). Although recent studies have shown that the redox reactions of Fe(III) oxide can be important for the mobilization and degradation of SOM (Grybos et al., 2007; Grybos et al., 2009; Hall and Silver, 2013), there is limited information available concerning how Fe-bound organic matter is released and metabolized during redox reactions. In this study, we investigated the release of hematite-bound organic matter during the reduction of Fe(III) by sodium dithionite, to elaborate the important processes for organic carbon (OC) during the redox reactions. Dithionite is one of the most commonly used Fe(III)-reducing agents for abiotic Fe reduction experiments (Eusterhues et al., 2014; Poulton & Canfield, 2004). We used a relatively low dithionite concentration to slow down the reduction kinetics and simulate natural environmental conditions. Using this abiotic reducing agent is very valuable and helpful for illustrating the governing processes for OC stability during the reduction dissolution of ferric Fe. The impact of the molecular structure of Fe-bound organic matter on the reductive release of OC was also analyzed.

2. Materials and Methods

2.1 Materials

Hematite was purchased from Fisher Chemical (Fisher Scientific, Pittsburgh, PA, USA) and used as received. The mineral phase was confirmed by X-ray diffraction (XRD) analysis. The surface area, determined by an AutoChem II 2920 (Micromeritics, Norcross, GA, USA), was 1.9 m²/g. Model SOM with a wide range of chemical compositions was generated using three sequential extractions of humic acids (HAs) from a peat soil (Supplementary Material, Table S1). Detailed information for extraction and characterization of the HAs is available in previous publications (Yang et al., 2011; Yang et al., 2012; Yang et al., 2013). Because the HAs were sequentially extracted from the same peat soil, this minimized the influence of other geochemical factors that might arise if using organic matter from different sources, such as different geological parent materials (Yang et al., 2011; Yang et al., 2012; Yang et al., 2013). Application of these previously extensively characterized HAs with a range of chemical composition provided us with an opportunity to study the role of different functional groups in the release and fate of OC during redox reactions. Specifically, quinones are thought to be important functional groups involved in the redox process (Lovley et al., 1996; Scott et al., 1998). There is a large variation in the content of quinone functional groups in the humic substances we used, which is beneficial for studying the role of quinones in the redox reactions of Fe and consequent processes for Fe-bound OC. For this study, the three HAs (HA1, HA2, and HA3) were dissolved in 0.5 M NaOH solution overnight and centrifuged at 3000 rpm to produce stock solutions with a concentration of 100 mg OC/L, as described in Adhikari and Yang (2015). All other chemicals used in this study were above analytical grade.

2.2 Synthesis of hematite-HA complexes and characterization for complexes

Hematite-HA complexes were made by sorbing HAs onto hematite under a range of pH values. Hematite and HA solution were mixed with a mass/volume ratio of 4 g/120 mL and shaken for 24 h to reach equilibrium. The mixtures were centrifuged at 10,000 rpm for 10 minutes and the hematite-HA complexes were collected and dried in an oven at 49°C for 40 h. Hematite-HA complexes were made at pH 5, 7 and 9, by adjusting with HCl or NaOH, to represent the wide range of pH values present in natural soils (Sparks, 2003). The pH influences the conformation of HA; when the pH is relatively low, more aggregate structures form. Hematite-HA complexes synthesized at different pH also can provide insights into the role of SOM conformation during redox reactions, which may influence the degradation and fate of the SOM. In total, nine different hematite-HA complexes were made in this study (i.e. three different HAs, each at pH 5, 7 and 9).

2.3 Dithionite reduction

Reduction of hematite was performed using the sodium bicarbonate, tri-sodium citrate and sodium dithionite (BCD) method (Holmgren, 1967). The reduction experiments were performed in duplicate. An aliquot (0.2 g) of dried hematite-HA complex was mixed with 15 mL buffer solution (pH 5) containing 1 M NaHCO_3 , 0.3 M $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ and 0.15 M $\text{Na}_2\text{S}_2\text{O}_4$. The mixtures were shaken at 100 rpm. At intervals over 960 minutes, samples were centrifuged at 10,000 rpm for 10 minutes and the supernatants were removed. The residue was rinsed with 15 mL of distilled, deionized water and centrifuged again. Rinse water was combined with previously collected supernatant for analysis of aqueous Fe. The residues were dried in an oven at 49°C until the weight was stable (change in weight < 0.1%) and then used for residual carbon concentration analysis with an elemental analyzer (EuroVector SpA, Milan, Italy). Pure hematite

was used as a control to quantify the reduction of Fe(III) by the BCD method. To analyze the non-reductive release of Fe-bound OC, hematite-HA complexes were reacted with a solution containing 1 M NaHCO_3 and 2.25 M NaCl (for equivalent ionic strength), following the same procedure for the reduction reaction. The release of carbon and Fe were analyzed. The influence of citrate sorption on the analysis of residual OC was tested by mixing pure hematite with 0.3 M $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$, 1 M NaHCO_3 and 0.45 M NaCl at pH 5, and hematite was collected at different times to analyze OC concentration.

2.4 Release kinetics of Fe and carbon

The release kinetics of Fe were obtained in two ways. Firstly, as sorbed organic matter contributes less than 1% of the total mass of the complexes, the mass of particles at different intervals was used to calculate the residual fraction of Fe. Secondly, the solution-phase Fe was analyzed using inductively coupled plasma with atomic emission spectroscopy (ICP-AES: Varian-Vista AX CCD, Palo Alto, CA, USA) by measuring the optical absorption of Fe at 259.9 nm. The reductive release kinetics of hematite-bound OC was measured simultaneously by analyzing the carbon concentration of the solid residue using an elemental analyzer (EuroVector SpA, Milan, Italy). We corrected the residual carbon fraction analyses by subtracting the sorption of citrate on hematite (2 mg OC/g hematite), determined in the control experiment (Supplementary Material, Fig. S1). The residual amount of carbon was calculated based on the carbon concentration and mass of particles collected after each period of reduction. These analyses were only possible for samples within 240 min of reaction, after which residual particles were too low in abundance to be accurately measured.

2.5 Release kinetics of aromatic carbon

Release kinetics for aromatic carbon were measured using a precipitation/re-dissolution method. To precipitate the HA released during the Fe reduction process, the pH of the supernatant collected from the reduction release experiments was reduced to below 1 by adding concentrated HCl. The HAs in the low-pH supernatant were then allowed to precipitate for 1 h and then centrifuged at 3,000 rpm for 10 min. The supernatant was discarded and the precipitated HA was rinsed with 10 mL of 1 M HCl to remove any residual Fe. The harvested HA was then re-dissolved in 8 mL of 1 M NaOH. The amount of aqueous aromatic OC was quantified by measuring the UV absorbance at 254 nm (UV_{254}) (Weishaar et al., 2003).

2.6 Carbon 1s near-edge X-ray absorption fine structure

Carbon 1s near-edge X-ray absorption fine structure (NEXAFS) analysis was performed for selected samples, i.e. original HA1, HA1-hematite synthesized at pH 7 and the HA1-hematite complexes after reduction for 240 min. A small amount of sample was mixed with distilled water and deposited onto a gold-coated silicon wafer, which was attached to a Cu sample holder with double-sided carbon tape. Samples were dried in a vacuum desiccator before analysis. Citric acid was used to calibrate the energy scale, assuming a value of 288.6 eV. The NEXAFS data were collected at the Canadian Light Source (Saskatoon, Canada) on the X-ray absorption spectroscopy endstation on the SGM beamline (Regier et al., 2007). The beamline uses a 45 mm planer undulator with an energy range between 250 and 2000 eV. The spot size on the beamline was 1000 μm $\times$ 100 μm . Data was collected using silicon drift detectors (SDD) and a titanium filter to reject higher order harmonics (Gillespie et al., 2015). The entrance and exit slit gaps were set to 249.9 μm and 25 μm . Slew scans (20 scans per sample) were acquired at the C 1s

edge from 270 to 320 eV over 20 s and on a new spot for each scan. Step scans were acquired from 280 to 320 eV at a 1 s dwell time and 0.1 eV step size. Normalization of the data involved collecting an I_0 by measuring the scatter of the incident beam from a freshly Au-coated Si wafer using an SDD. The scatter in the sample data was removed by adjusting the pre-edge baseline to near zero before normalizing with the I_0 (Gillespie et al., 2015).

2.7 Electron paramagnetic resonance analysis

Electron paramagnetic resonance (EPR) spectra were used to analyze the semi-quinone radicals in HAs, which were dissolved in 1 M NaOH and scanned for organic radicals using a Bruker EMX PremiumX-ER075 EPR (Billerica, MA, USA). The wave frequency was set at 9.3 GHz, power at 2.00 mW, attenuation at 20.0 dB, and the spectra were analyzed using Xenon 1.1b 44. To reduce the polarity effect due to water, 50 μ L of the HA solution was collected in a borosilicate glass capillary tube (Blaubrand micropipette, Wertheim, Germany) and sealed with glue stick (Scotch, 3M) at both ends. The capillary tube was then loaded into an EPR tube (Norell Inc., Landisville, NJ, USA) and scanned. To measure the background signal, an empty capillary tube sealed with glue was scanned.

2.8 Scanning electron microscope with energy dispersive X-ray spectroscopy (SEM-EDS)

Residual particles of hematite-HA complexes were directly loaded to a double-sided carbon tape on a silicon chip for analysis using a Hitachi S-4700 II scanning electron microscope (Hitachi, Ltd. Tokyo, Japan) with energy dispersive X-ray spectrometer analysis (EDS). No coatings were performed on the samples. The accelerating voltage was 20 kV. The element profiles were determined based on the surface scan with scanning area of 0.62-10.3 μm^2 . Each sampling area

was scanned for at least 45 seconds, and each sample was scanned for 3 different areas. Atomic fractions obtained from EDS analysis were used to compare their surface abundance.

2.9 Kinetics Modeling

We used empirical models to simulate the release kinetics of carbon and Fe. The release kinetic of carbon was fitted with an empirical model:

$$f_c = 1 - Z_c * (1 - \exp(-k_c * t)) \quad (1)$$

where f_c is the fraction of residual carbon remaining as a function of time, k_c is the release rate constant (min^{-1}), t is time (min), and Z_c is a unitless constant reflecting the steady level of residual carbon fraction.

Similarly, the residual Fe fraction was fitted using:

$$f_{Fe-res} = \exp(-k_{Fe-res} * t) \quad (2)$$

where f_{Fe-res} is the fraction of residual Fe remaining as a function of time, and k_{Fe-res} is the release rate constant (min^{-1}).

The solution phase Fe release fraction was fitted using:

$$f_{Fe-sol} = 1 - \exp(-k_{Fe-sol} * t) \quad (3)$$

Where f_{Fe-sol} is the fraction of Fe released to the solution phase as a function of time, and k_{Fe-sol} is the release rate constant (min^{-1}).

Similarly, the release of aromatic carbon was simulated using:

$$UV_{254} = UV_{254s} * (1 - \exp(-k_{aro} * t)) \quad (4)$$

Where UV_{254} is the UV absorbance at 254 nm as a function of time, UV_{254s} is the steady UV absorbance at 254 nm, and k_{aro} is the aromatic carbon release rate constant (min^{-1}).

All the empirical model fitting for the kinetics analyses were performed using MATLAB 14a.

3. Results and Discussion

3.1 Reductive release kinetics of Fe and OC

Over 90% of Fe in the hematite-HA complexes was reduced within 960 min (Fig. 1). The residual mass of hematite gradually decreased as a result of reduction and release by dithionite, while in the control experiment with only bicarbonate, hematite was stable over the experimental duration (Supplementary Material, Fig. S2). The residual mass of hematite decreased in the active experiments, while there was a concomitant increase in the concentration of aqueous Fe, with final recovery of $90\pm 20\%$ of the initial Fe (in hematite) in the form of aqueous Fe. Both the decrease in the residual mass of hematite and the increase in aqueous Fe can be fitted with first-order models (Table 1, $R^2=0.90-0.97$). Based on the data for the decrease in the residual mass of hematite, the half-life for the reduction of Fe was 73-95, 72-104 and 53-83 min for hematite-HA1, hematite-HA2, and hematite-HA3, respectively, and 84 min for pure hematite (Supplementary Material, Fig. S3). The difference in the half-life for Fe reduction of the hematite-HA complexes compared to pure hematite was below 20% for most of the samples, except hematite-HA3 synthesized at pH 5 and 7, indicating that the reduction of Fe(III) was affected by the sorbed HA to a minor degree. Previous studies showed that humic substances can increase the microbial reduction rate of Fe (Lovley et al., 1996; Roden et al., 2010; Scott et al., 1998), although Eusterhues et al. (2014) found that the sorption of humic substances onto ferrihydrite decreased the abiotic and biotic reduction rate constant of Fe(III) by a factor of up to ten times. The relatively minor effect of HA on Fe reduction rates in our study was due to the strong reduction induced by dithionite and the small amount of organic matter sorbed by hematite.

The decrease in the residual fraction of OC vs. time is illustrated in Fig. 1, for which the data is restricted to the first 240 min of the reaction. Control experiments with only bicarbonate showed no dissolution of Fe or release of OC, indicating that the release of hematite-bound OC was mainly due to the reductive reaction. The release of OC was asynchronous with that of Fe with OC released more rapidly than Fe initially, and there were significant differences among the OC release rates under different conditions. For example, OC was released rapidly in the first 15 minutes, at a faster rate than for Fe release for the hematite-HA1 synthesized at pH 5 (Fig. 1a). The residual amount of OC decreased to 37.8% of the original concentration, but then remained relatively stable between 15 and 240 min. The rapid initial release of OC is important for the biogeochemical cycle of OC, especially considering that the reducing conditions are brief for some natural media, such as surface soils. The rapid initial release of OC can make it more bioavailable and facilitate its consequent metabolism and degradation by indigenous microorganisms. In contrast, for hematite-HA3 synthesized at pH 9 (Fig. 1i) the OC was released gradually and corresponds closely to the release of Fe. Values for the initial release rate constants for OC ranged from 0.11 to 1.49 min⁻¹, and the final values of residual OC (1-Z_c) were 4.6-58.2% (Table 1). The discrepancies between the rate constant for the release of Fe and OC suggest that the OC release was governed not only by the reduction of Fe, but also by the sorptive interactions between HA and hematite. Differences in reductive release rates of OC for different hematite-HA complexes suggest that the OM molecular structure influences its reductive release.

Fig. 1 Release kinetics of Fe and C in hematite-humic acid (HA) complexes in the reduction reaction with dithionite. pH values indicate the pH at which the complex was prepared. The time-dependent release fraction (black circle) and residual fraction (open circle) of Fe as well as the residual fractions of carbon in active experiments (red diamond) and in control experiments (grey square) are presented. The lines indicate the fitting results from empirical models. The error bars represent the standard error of duplicate analyses. The error bars fall within the symbol for points where error bars are not visible.

Table 1: Fitted parameters for the release of Fe and OC from hematite-HA complexes using empirical equations (eq. 1-3).

Humic Acid	pH	k_{Fe-res} ($\times 10^{-3} \text{ min}^{-1}$) ^a	Std ^b	$t_{1/2}$ ^c (min)	k_{Fe-sol} ($\times 10^{-3} \text{ min}^{-1}$) ^d	Std	$t_{1/2}$ (min)	k_c (min^{-1}) ^e	Std	Z_c ^f	Std
HA1	5	7.5	0.9	93	10.1	0.3	69	1.49	0.000	0.622	0.000
HA2	5	8.0	0.8	87	9.5	1.9	73	0.977	N ^g	0.418	0.098
HA3	5	10.9	3.0	64	8.9	2.8	78	0.775	0.000	0.685	0.000
HA1	7	9.5	1.2	73	9.8	1.7	71	0.231	0.191	0.954	0.002
HA2	7	9.6	1.3	72	8.4	3.5	82	0.224	0.207	0.715	0.035
HA3	7	13.0	1.2	53	15.1	3.4	46	0.541	N	0.527	0.050
HA1	9	7.3	1.0	95	6.0	1.5	116	0.213	0.178	0.874	0.043
HA2	9	6.7	0.8	104	6.2	1.2	112	0.054	0.010	0.795	0.037
HA3	9	8.3	1.3	83	9.0	2.1	77	0.011	0.003	0.781	0.101
Hematite ^h		8.2	0.5	84							

a K_{Fe-res} : release rate constant obtained for the kinetics of residual fraction of Fe;

b Std: standard deviation;

c $t_{1/2}$: half-life for Fe reduction and release;

d K_{Fe-sol} : release rate constant obtained for the kinetics of dissolved fraction of Fe;

e K_c : initial release rate constant for carbon release;

f Z_c : the stable residual fraction of carbon;

g N: no good constrains were obtained for this parameter;

h Pure hematite.

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284 3.2 Impact of physiochemical properties of HA on the reductive release of OC

285 The chemical composition of HAs, and specifically the nature and abundance of the functional
286 groups, can be an important factor governing the distribution of organic matter on the hematite
287 surface and the reductive release of Fe-bound organic matter. The release rate constants (K_c) for
288 hematite-bound HA correlate positively with the aromatic carbon fraction in HA for complexes
289 made at pH 5 and pH 9 (both with Spearman Correlation coefficient = 1), while the released

fraction up to the stable stage (Z_c) of OC correlates positively with the aromatic carbon fraction in HA for complexes made at pH 7 and pH 9 (both with Spearman Correlation coefficient = 1) (Supplementary Material, Fig. S4). These relationships indicate the importance of aromatic carbon in the release of Fe-bound organic matter, with a higher aromatic fraction leading to the more rapid release of OC and a higher fraction of final release of OC. When pH for complex synthesis increased, the K_c decreased, but Z_c increased. The influence of pH on K_c and Z_c is probably due to the nature of the interaction between the organic matter and hematite. For HAs used in this study, the point of zero charge (pZc) was 3.3-5.1 (Wang et al., 2011), so a change in pH from 5 to 9 for complex synthesis would increase the surface charge and electronic double layer potential of HAs (Pan et al., 2008). Partially because of the change in organic matter surface charge, the increase in pH would inhibit the aggregation of organic matter and decrease the size of organic matter aggregates (Pan et al., 2008). Our findings suggest that the hematite-bound organic matter with smaller aggregation was released less during the initial stage, as shown by a decrease in K_c .

To further investigate the role of aromatic carbon in the release of Fe-bound organic matter, the reduction-release kinetics for aromatic organic matter were directly monitored by measuring UV_{254} for precipitated/re-dissolved samples. The precipitate of most samples had a dark brown color characteristic of HA, while visible precipitates were not formed for the hematite only control (Supplementary Material, Fig. S5). UV_{254} has been suggested to be an excellent indicator for aromatic carbon in the solution phase (Weishaar et al. 2003). UV_{254} increased rapidly within the first 60 minutes and then remained relatively steady at a value between 0.25 and 0.65, while UV_{254} for control experiments with pure hematite produced a minor absorbance value of

0.07±0.03 (Fig. 2). In this study, the concentration of dissolved organic matter was relatively low, which precluded further quantitative analysis for chemical composition, such as nuclear magnetic resonance (NMR) spectroscopic analysis. Kinetic analysis of the UV₂₅₄ data indicates that the aromatic organic matter was initially released relatively rapidly and then the values of UV₂₅₄ remained relatively steady after approximately 60 minutes. The kinetics for UV₂₅₄ can be fitted with an empirical model (eq 4, Fig. 2, Supplementary Material, Table S2), and yield initial release rate constants of 0.035-0.080, 0.083-0.11 and 0.013-0.080 min⁻¹ for hematite-HA1, hematite-HA2, and hematite-HA3, respectively. The time for the UV₂₅₄ to reach 90% of the final stable values ranged from 21 to 87 min.

Fig. 2 Time-dependent UV_{254} measured for samples recovered from precipitation/re-dissolution for released organic matter from hematite-HA complexes (circle) and pure hematite (triangle). Lines represent the fitting results using empirical models (eq 4). Panel a, d and g are for complexes of hematite-HA1, HA2 and HA3 made at pH 5; Panel b, e and h are for complexes of hematite-HA1, HA2 and HA3 made at pH 7; Panel c, f and i are for complexes of hematite-HA1, HA2 and HA3 made at pH 9. The error bars represent the standard error of duplicate analyses. The error bars fall within the symbol for points where error bars are not visible.

By comparing the fitted release of total OC and aromatic carbon, we can derive the relative release of aromatic carbon:

$$f_{aro-R} = \frac{1 - \exp(-k_{aro} * t)}{Z_C * (1 - \exp(-k_C * t))} \quad (5)$$

where f_{aro-R} is an index for the relative release rate of aromatic carbon vs. total OC for hematite-bound HAs. For most samples, except hematite-HA1 at pH 7, f_{aro-R} is greater than 1, with values of 1.05 to 1.61, 1.26 to 2.40, and 1.30 to 1.90 at 240 min for complexes of hematite-HA1, HA2 and HA3, respectively (Fig. 3). The released fraction of aromatic carbon rapidly increased, and approached 0.90 within 25-49 min, 25-28 min, and 20-201 min for hematite-HA1, hematite-HA2, and hematite-HA3 complexes, respectively. At 240 minutes, the released fraction of total carbon achieved 0.62-0.95, 0.41-0.79 and 0.52-0.71 for hematite-HA1, hematite-HA2, and hematite-HA3 complexes, respectively. These results demonstrate that aromatic carbon was more rapidly released compared to the rest of the HA components, consistent with the relationships between the release rate constant and fraction of aromatic carbon in HA (Supplementary Material, Fig. S4).

Fig. 3 The relative release of aromatic carbon compared to the total organic carbon (f_{aro-R}). Line of $f_{aro-R}=1$ as reference.

The changes in the chemical composition of Fe-bound OC upon the reduction were also analyzed by C 1s NEXAFS analysis. The HA1 C 1s spectrum exhibited peaks at 285.1, 286.6 and 288.6 eV corresponding to aromatic, phenolic and carboxylic/amide, respectively (Lehmann et al., 2009; Lehmann et al., 2008; Solomon et al., 2005). Both phenolic and carboxylic/amide are in reference to non-aromatic carbon, although the content of phenolic carbon was related to that of aromatic carbon (Yang et al., 2013). The spectrum is similar to that reported for humic acid (Christl and Kretzschmar, 2007; Schumacher et al., 2005). The ratio of the aromatic carbon and the phenolic carbon peaks (height) to the carboxylic peak was 0.29 and 0.39, respectively. The spectrum of the hematite-HA1 complex synthesized at pH 7, before reduction, also contained similar peaks and ratios to those of the HA1. After reduction of the hematite-HA complex for 240 min, the intensity of the aromatic and phenolic peaks decreased substantially compared to the carboxylic peak, with ratios of 0.11 and 0.19, respectively (Fig. 4). This change supports the rapid release of aromatic carbon, but the ratios were also influenced by the sorption of citrate, which disabled further more accurate analysis.

Fig. 4 C1s NEXAFS spectra for HA1, HA1-hematite complexes (synthesized at pH 7) and HA1-hematite complexes (synthesized at pH7) after 240-min reduction using BCD method. Peaks at 285.1, 286.6 and 288.6 eV correspond to aromatic, phenolic and carboxylic carbon, respectively.

3.3 Mechanism for the preferential release of aromatic carbon

The relatively faster release of aromatic carbon in comparison with other functional groups is probably due to the specific interactions between aromatic functional groups and hematite. Our previous study determined that organic matter was sorbed onto the hematite surface through a multiple-layer heterogeneous coating instead of a mono-layer homogeneous coating, and aromatic carbon was likely distributed on the outer layer as a result of steric effects of rigid aromatic structures (Adhikari and Yang, 2015). Hence, it is potentially easier to release aromatic carbon on the outer layer as compared to other non-aromatic components residing in the inner layer. In addition to steric effects, the rapid release of aromatic organic matter may also be

related to the involvement of aromatic functional groups in the redox reaction. In particular, previous studies have demonstrated the importance of quinone in the electron-accepting and electron-donating processes of HAs (Newman and Kolter, 2000; Roden et al., 2010; Scott et al., 1998) and the quinone-related reaction may contribute to the release of aromatic carbon. For determination of the aromatic and phenolic fraction in HA, a chemical shift of 109-145 ppm and 145-160 ppm for ^{13}C -NMR spectra were assigned as aromatic and phenolic carbon, respectively, but the chemical shift for quinone (140-160 ppm) overlaps with this range (McDonald et al., 1977; Simpson et al., 2011; Wang and Xing, 2007). In addition, EPR analyses for our HA samples show significant signals for all three HAs at pH 11 (Fig. 5), with g values of 2.00442, 2.00458 and 2.00295 for HA1, HA2 and HA3, respectively. Previous studies indicate g values are a good index to distinguish different types of radicals, with $g < 2.003$ for carbon-centered radicals and $g > 2.004$ for oxygen-centered (Barclay and Vinqvist, 1994; Dellinger et al., 2007; Liao et al., 2014; Senesi and Steelink, 1989). The g is 2.0028 and 2.0026 for graphitic carbon and polycyclic aromatic carbon radicals, while it is normally above 2.004 for semi-quinone radicals (Delhaes and Marchand, 1968; Szent-Györgyi and Isenberg, 1960). The results imply that HA1 and HA2 mainly contained oxygen-centered radicals, while HA3 contained carbon-centered radicals. Carbon-centered radicals can also be contributed by semi-quinone groups, because of the resonance effects and lower activation energy for carbon-centered semi-quinone radicals compared to those oxygen-centered (Dellinger et al., 2007). As the same amount (20 mg) of HA1-3 was used for this EPR analysis, the integrated area under EPR spectra is an indicator for the concentration of semi-quinone radicals (Yang et al., 2013). The value was 9.5, 3.5 and 0.9 for HA1, HA2 and HA3, with the same rank as for their aromatic carbon and consequently the release rate constant (k_c) of OC at pH 5 and 9 (Supplementary Material, Fig.

S4). A probable explanation for this pattern is that the quinone content was linearly related with the occurrence level of semi-quinone radicals (Scott et al., 1998). The quinone and semi-quinone can potentially be reduced by dithionite, and reduction products (semi-quinone and hydroquinone) would reduce Fe(III). Such redox reactions would likely weaken the association between quinone functional groups and hematite, and facilitate the release of quinones, part of aromatic functional groups. Further investigations will be necessary to elucidate the detailed mechanism responsible for this behavior.

Fig. 5 First derivative electron paramagnetic resonance (EPR) spectra for freshly dissolved HA1, HA2 and HA3.

Finally, the organic matter coating on hematite surface was analyzed using SEM-EDS analysis. The EDS-based surface C/Fe ratio was taken as a semi-quantitative indicator for the relative occupation of organic matter on the hematite surface. At pH 7, the time-dependent change of the surface C/Fe ratio was different for the three HAs (Supplementary Material, Fig. S6 and Table S3). For HA1, the surface C/Fe ratio rapidly decreased from 2.1 to 0.4 within the first 15 min., and then remained approximately constant. This likely indicates the relative coverage fraction of

organic matter on hematite surface decreased initially due to the more rapid release of organic matter compared to Fe oxide, but did not change after the first 15 min. For HA2, the surface C/Fe ratio increased to a peak value at 60 min, and then decreased, while the surface C/Fe ratio for HA3 slowly increased over time until the end of the experiment. These observations imply that the surface coverage of organic matter possibly increased for HA2 and HA3 (peak C/Fe for HA2 at 60 min., maximum C/Fe for HA3 at the end of the experiment) even though the release of carbon was faster than Fe. We suggest that this may result from the removal of surface organic matter on the top layer of a multiple-layer coating (having little effect on the areal coverage of adsorbed OC), while the total area of exposed Fe decreased due to reductive dissolution. Previous studies demonstrated that the molecular size increased from HA1 to HA2 to HA3, with median molecular weights of 1580, 2500 and 4000 D respectively (Yang et al., 2012). The larger molecular size of HA can likely lead to the coating of more layers, which can be responsible for the increased surface C/Fe parallel to the more rapid release of bulk carbon compared to iron. More accurate abundance and distribution analysis of surface coating and morphology of HA on hematite surfaces will be required in further studies, as we acknowledge the limit of SEM-EDS in quantitative analysis and also the relatively large uncertainty in our results partially because of the technical limits of the SEM-EDS method.

4. Conclusion and Implications

Mineral-organic matter complexes are ubiquitous in soil and sediment environments, and play an important role in regulating the stabilization of organic carbon. Reductive release of adsorbed organic matter from Fe(III) oxide surfaces represents an important mechanism to destabilize and mobilize adsorbed organic carbon. During reduction experiments, adsorbed organic matter was

released more quickly than Fe to the aqueous phase (Fig. 6). Adsorbed aromatic organic matter was more rapidly released compared to total adsorbed organic matter, illustrating that the specific chemical composition of adsorbed organic matter on Fe oxides, particularly the type and concentration of functional groups, is critical for understanding and predicting the stabilization vs. de-stabilization of organic carbon adsorbed onto Fe oxides.

Fig. 6 A conceptual scheme describing the comparison of Fe reduction and OC release during the abiotic reduction.

These results have several implications for the dynamics of Fe and C behavior in soils. First, we found the release of C was asynchronous – it was not linearly related to the reduction and release of Fe. Especially during the initial stage, the release of C was more rapid than Fe, indicating the importance of Fe reduction to the stability of C. The integration of redox reactions into biogeochemistry models of C is important for accurately predicting the residence time of C in soil. Second, the release of C is closely dependent on the chemical composition of its functional groups. Aromatic C is preferably released compared to other components. Prediction for the

persistence of C during redox reactions is tied to the chemistry of C. Relative persistence of aliphatic C during the redox reactions, consistent with our recent publication (Adhikari et al., 2015), provided new insight into the occurrence and persistence of aliphatic C in natural environments. Third, hematite, as an important iron oxide minerals in soil and, unlike ferrihydrite, does not go through crystal transformation during the reduction reaction (Biederman et al., 2013; Kappler et al., 2014). Thus, hematite is an environmentally relevant model mineral that can be used for investigating the linkage between the release of C and reduction of Fe without mineral transformations that may confound the data.

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