

1 **Isotopic variation of dissolved and colloidal iron and copper in a carbonatic**  
2 **floodplain soil after experimental flooding**

3  
4 **Charirat Kusonwiriya<sup>a</sup>, Moritz Bigalke<sup>a\*</sup>, Florian Abgottspon<sup>a</sup>, Marina Lazarov<sup>b</sup>,**  
5 **Stephan Schuth<sup>b</sup>, Stefan Weyer<sup>b</sup>, Wolfgang Wilcke<sup>c</sup>**

6  
7 <sup>a</sup>Geographic Institute, University of Berne, Hallerstrasse 12, 3012 Berne, Switzerland

8 <sup>b</sup>Institute of Mineralogie, Leibniz University Hannover, Callinstrasse 3  
9 30167 Hannover, Germany

10 <sup>c</sup>Institute of Geography and Geoecology, Karlsruhe Institute of Technology (KIT), Reinhard-  
11 Baumeister-Platz 1, 76131 Karlsruhe, Germany.

12 \*Corresponding author: Moritz Bigalke, phone: +41(0)316314055,  
13 [moritz.bigalke@giub.unibe.ch](mailto:moritz.bigalke@giub.unibe.ch)

14  
15 **Abstract**

16 Many floodplain soils worldwide are contaminated by present and past industrial and mining  
17 activities. During flooding redox potential decreases, triggering the release of dissolved and  
18 colloidal metals. We used an anaerobic microcosm incubation to simulate flooding of a  
19 carbonate-rich floodplain soil for 40 days. Soil solution samples were extracted to determine  
20 the release of dissolved (<0.02  $\mu\text{m}$ ) and colloidal fractions (0.02 - 10  $\mu\text{m}$ ). We analyzed stable  
21 isotope ratios of colloidal and dissolved Fe and Cu representing two groups of metals with  
22 different release behavior; release of Fe was steadily increasing, while Cu peaked sharply  
23 after flooding and decreased afterwards. The temporal trend of  $\delta^{56}\text{Fe}$  values of total Fe in

24 solution indicated dissimilatory iron reduction. The apparent isotopic fractionation between  
25 dissolved and colloidal Fe ( $\Delta^{56}\text{Fe}_{\text{dissolved-colloidal}} = \delta^{56}\text{Fe}_{\text{dissolved}} - \delta^{56}\text{Fe}_{\text{colloidal}}$ ) varied between  
26  $0.31 \pm 0.04\text{‰}$  and  $-1.86 \pm 0.26\text{‰}$ . Low  $\delta^{56}\text{Fe}_{\text{colloidal}}$  ( $-1.16 \pm 0.04\text{‰}$ ) values on day 4 of the  
27 experiment suggested colloid formation by precipitation of dissolved Fe, while the strong  
28 temporal variation in  $\Delta^{56}\text{Fe}_{\text{dissolved-colloidal}}$  indicated subsequent changes in colloid mineralogy,  
29 sorption to soil components and/or electron transfer-atom exchange. The variations in  $\delta^{65}\text{Cu}$   
30 values ( $\Delta^{65}\text{Cu}_{\text{dissolved-colloidal}}$  from  $0.81 \pm 0.03\text{‰}$  to  $1.58 \pm 0.09\text{‰}$ ) are probably linked to  
31 changing oxidation state of colloidal Cu. While at the beginning of the experiment colloidal  
32 Cu and solid soil Cu exchange, these systems decouple after the onset of sulfate reduction in  
33 the second half of the experiment. The experimental results fit well to findings from  
34 redoximorphic soils described in the literature and highlight the importance of colloids for  
35 metal release and the isotopic pattern in carbonatic soils.

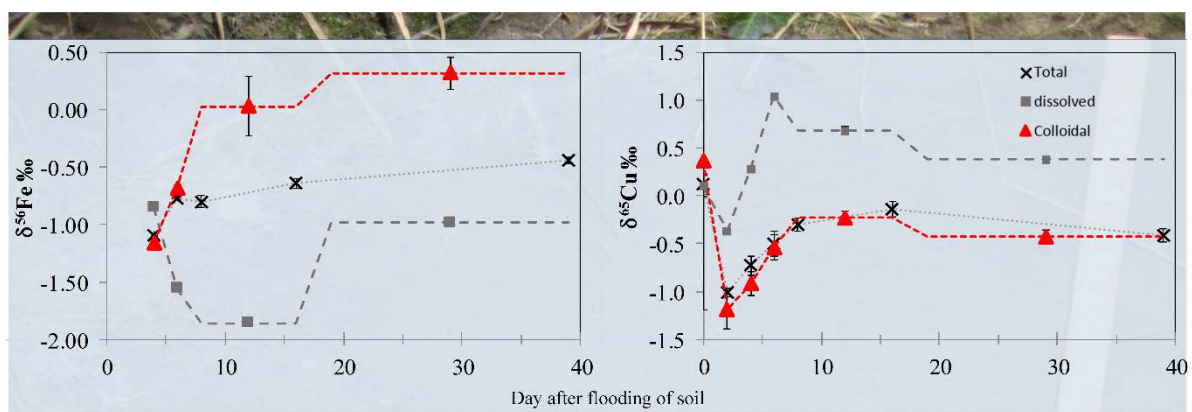
36 **Keywords: Copper, Iron, Soil, Redox Processes, Colloids, Stable Metal Isotopes**

37

### 38 **Highlights**

- 39 • Colloidal Fe precipitates from dissolved Fe in soil solution and does not originate from  
40 the mobilization of solid Fe minerals.
- 41 • The  $\delta^{56}\text{Fe}$  value of colloidal Fe changes with time indicating changes in mineralogy or  
42 electron transfer-atom exchange.
- 43 • Colloidal and solid soil Cu exchange occurs until sulfate reduction starts, afterwards  
44 colloidal Cu decouples from solid soil and colloids aggregate.
- 45 • The  $\Delta^{65}\text{Cu}_{\text{dissolved-colloidal}}$  values are probably driven by the redox state of colloidal Cu.
- 46 • Experimental findings agree well with field observations.

47

48 **Graphical Abstract**

49

50

51  
52

## 1. Introduction

53 Present and past industrial and mining activities release contaminants into rivers, which  
54 continuously accumulate in floodplain soils (Burton et al., 2008; Frohne et al., 2011;  
55 Hindersmann and Mansfeldt, 2014). Depending on the frequency of flooding, metals might be  
56 remobilized and contaminate ground and surface waters (Du Laing et al., 2007; Hindersmann  
57 et al., 2014; Mansfeldt and Overesch, 2013). The mobility of metals mainly depends on  
58 organic matter concentrations and composition, soil mineral composition, redox conditions,  
59 and carbonate concentrations (Du Laing et al., 2009). When soils are flooded, the redox  
60 potential ( $E_h$ ) decreases and Fe and Mn (oxyhydr)oxides are dissolved in many soils releasing  
61 associated trace elements (e.g., As, Ba, Co, Cr, V; Abgottspon et al., 2015; Hindersmann and  
62 Mansfeldt, 2014; Weber et al., 2009b). In some soils with permanent water saturation, the  
63 pool of reducible Fe and Mn is depleted and no further mobilization occurs under anoxic  
64 conditions. The release kinetics depends strongly on crystallinity of Fe and Mn minerals and  
65 microbial community composition in the soil (Lovley, 1991; Schuth et al., 2015). In  
66 carbonatic soils, the pH is buffered by the carbonic acid-hydrogen carbonate buffer system  
67 and proton release during formation of minerals like siderite and rhodochrosite (Abgottspon et  
68 al., 2015; Ponnampereuma, 1972). Furthermore, changes to anoxic conditions may cause  
69 microbial formation of reduced metal colloids (e.g.,  $Cu^0$ , Weber et al., 2009b). When  $E_h$   
70 decreases, microbial sulfate reduction may be initialized and mobility of metals can be limited  
71 by the formation of or co-precipitation with sulfides (Borch et al., 2010; Weber et al., 2009a).  
72 However, sulfate reduction may also favor the release of metal-sulfide colloids of some  
73 elements (e.g., Ag, Cd, Cu, Hg, Pb) resulting in enhanced metal mobility during several days  
74 after flooding (Abgottspon et al., 2015; Hofacker et al., 2013; Weber et al., 2009b). The  
75 formation of sulfides is limited by the available sulfate content and controlled by the  
76 solubility of the corresponding metal sulfides (Weber et al., 2009a). Beside the formation of

77 organic, biomineralized and sulfide colloids, also colloids of Fe and Mn minerals may favor  
78 trace element transport (Hasselov and von der Kammer, 2008). The colloids may form  
79 because of precipitation of siderite and rhodochrosite in carbonatic soils or the precipitation of  
80 mixed valence (oxyhydr)oxides (e.g., magnetite or green rust; Ratering and Schnell, 2000;  
81 Tadanier et al., 2005; Taylor, 1980). Another colloid formation process is the partial reduction  
82 of ferrihydrite by microorganisms which may initialize deflocculation and release of the  
83 remaining ferrihydrite as colloids along with associated non-Fe elements (Tadanier et al.,  
84 2005). When soil flooding ends and redox conditions change back from anoxic to oxic,  
85 sulfides will be oxidized, releasing metals like Cd, Cu and Zn to pore waters, which may  
86 (co)precipitate with (oxyhydr)oxides (Contin et al., 2007; Du Laing et al., 2007; Frohne et al.,  
87 2011).

88 Abgottspon et al. (2015) differentiated trace metals into two groups with different  
89 release behavior in water-saturated soils. When  $E_h$  decreased (from 300 mV to 0 mV), Group  
90 I metals (Ba, Co, Cr, Ni, and V) were constantly released during 40 days, predominantly in  
91 dissolved form (<50 % as colloids) closely correlated with the release of Fe and Mn by  
92 reductive dissolution of (oxyhydr)oxides. The increase in colloidal concentrations of Group I  
93 metals is probably attributable to coprecipitation of dissolved metals with and sorption to  
94 colloidal Fe and Mn minerals. Group II metals (Ag, Cd, Cu and Pb) were rapidly released  
95 after 2-6 days of flooding, strongly dominated by the colloidal fraction (>80%; 0.02-10  $\mu\text{m}$ ).  
96 In this study, we focus on the stable isotope ratios of Fe and Cu as representatives of Group I  
97 and Group II metals, respectively, to further explore the mechanisms underlying the observed  
98 differences in the release behavior of the two metal groups.

99 The stable isotope ratios of Fe and Cu bear a high potential to investigate long-term  
100 biogeochemical processes in soils, especially redox processes (Babcsanyi et al., 2014; Bigalke  
101 et al., 2013; Bigalke et al., 2010b; Mansfeldt et al., 2012; Wiederhold et al., 2007a). Iron is an  
102 important driver of biogeochemical processes and trace metal behavior in soils. Changing the

103 oxidation state of Fe results in marked Fe isotope fractionation causing variations in Fe  
104 isotopic composition among Fe pools in anoxic soils with  $\delta^{56}\text{Fe}$  values ranging from -5‰ to  
105 +1‰ (Johnson et al., 2008). Under anoxic conditions, dissimilatory iron reduction (DIR) by  
106 bacteria initializes three main Fe reaction pools, i.e.  $\text{Fe}^{2+}_{(\text{aq})}$ ,  $\text{Fe}^{2+}_{(\text{sorb})}$  and  $\text{Fe}^{3+}_{(\text{reac})}$ . Some of  
107 the dissolved  $\text{Fe}^{2+}_{(\text{aq})}$  may sorb to the oxide surface as  $\text{Fe}^{2+}_{(\text{sorb})}$ . Electron transfer between  
108  $\text{Fe}^{2+}_{(\text{sorb})}$  and  $\text{Fe}^{3+}$  creates a reactive layer of  $\text{Fe}^{3+}$  at the oxide surface ( $\text{Fe}^{3+}_{(\text{reac})}$ ) and is  
109 associated with  $^{56}\text{Fe}$  enrichment in the solid phase. Subsequently, DIR releases isotopically  
110 light Fe into solution while heavy Fe isotopes are preferentially adsorbed on Fe oxide surfaces  
111 ( $\Delta^{56}\text{Fe}_{\text{mineral-solution}}$  up to 4‰; Frierdich et al., 2014; Icopini et al., 2004; Mikutta et al., 2009;  
112 Teutsch et al., 2005). The apparent isotope fractionation between  $\text{Fe}^{2+}_{(\text{aq})}$  and  $\text{Fe}^{3+}_{(\text{reac})}$  is  
113 identical regardless of bacteria species or type of oxides yielding  $\Delta^{56}\text{Fe}_{\text{Fe(II)-Fe(III)reac}}$  values of  
114  $\sim -3\text{‰}$  (Crosby et al., 2007). When the reduction continues, increased  $\text{Fe}^{2+}$  concentrations in  
115 solution result in a shift to a heavier Fe isotopic composition of dissolved Fe because of  
116 isotopic pool-size effects. This fractionation depends on the relative proportion of the three  
117 main reactive Fe pools (Crosby et al., 2007). Schuth et al. (2015) conducted incubation  
118 experiments under controlled  $E_h$  conditions and found strong variations in bulk  $\delta^{56}\text{Fe}$  values  
119 in soil solutions of a surface soil horizon, while a permanently anoxic lower soil horizon  
120 showed no Fe release and no response of  $\delta^{56}\text{Fe}$  values to flooding, probably because of a more  
121 stable crystalline Fe oxide mineralogy or an absence of Fe-reducing bacteria. However, at  
122 constant  $E_h$  a steady state of  $\delta^{56}\text{Fe}$  values and dissolved total Fe was observed by Schuth et al.  
123 (2015) for the topsoil because of the balance of Fe release and removal from solution. Garnier  
124 et al. (2017) and Thompson et al. (2007) found Rayleigh-type fractionation during DIR in  
125 redox-influenced soils but smaller fractionation compared to the experimentally determined  
126 fractionation factors, because of additional processes occurring in soil (e.g. organic  
127 complexation).

128 Ilina et al. (2013a) studied iron isotope variations in river, lake and mire waters and  
 129 soil solutions of different filtration sizes reaching from 100  $\mu\text{m}$  to  $<1$  kDa. The  $\delta^{57}\text{Fe}$  values  
 130 of mire waters (0.12 to 0.66 ‰) and soil solutions (-0.20 to -0.42 ‰) showed no systematic  
 131 differences except for the  $<1$  kDa fraction, which was the lightest (-0.83 to -0.91 ‰). River  
 132 waters became constantly heavier (up to 4.2 ‰) with decreasing pore size, which was  
 133 explained by stronger Fe binding of low molecular weight organic compounds compared to  
 134 bigger particles like Fe (oxyhydr)oxides (Ilina et al., 2013a). A similar observation was made  
 135 by Schuth and Mansfeldt (2016) for stagnant water, where smaller particles indicated a slight  
 136 enrichment in  $^{56}\text{Fe}$ . Furthermore, other mechanisms can induce Fe isotope fractionation.  
 137 Heavy Fe isotopes are preferentially adsorbed or precipitated on the bacterial cell surface  
 138 ( $\Delta^{56}\text{Fe}_{\text{cell-solution}}$  up to 1.7‰) and complexed by organic ligands ( $\Delta^{56}\text{Fe}_{\text{complex-solution}}$  of 0.6‰;  
 139 Brantley et al., 2001; Dideriksen et al., 2008; Morgan et al., 2010; Mulholland et al., 2015).  
 140 Precipitation of siderite caused a lighter Fe isotope ratio in the siderite ( $\Delta^{56}\text{Fe}_{\text{dissolved-siderite}} =$   
 141 0.0-0.9‰; Johnson et al., 2005; Wiesli et al., 2004). While kinetic FeS precipitation caused an  
 142 enrichment of light Fe in the precipitate ( $\Delta^{56}\text{Fe}_{\text{Fe(II)aq-FeS}} = 0.85 \pm 0.30$ ‰; Butler et al., 2005),  
 143 precipitation of mackinawite at equilibrium conditions caused oppositional fractionation of  
 144  $\Delta^{56}\text{Fe}_{\text{Fe(II)aq-mackinawite}} = -0.32 \pm 0.29$ ‰ to  $-0.64 \pm 0.36$ ‰ (Guilbaud et al., 2011; Wu et al., 2011).  
 145 The precipitation of magnetite similarly causes an enrichment of heavy isotopes in the  
 146 precipitates ( $\Delta^{56}\text{Fe}_{\text{Fe(II)aq-magnetite}} = -1.34 \pm 0.11$ ‰ to  $-1.56 \pm 0.20$ ‰; Frierdich et al., 2014;  
 147 Johnson et al., 2005).  
 148 Sorption of Cu to Al and Fe (oxyhydr)oxides causes an enrichment of heavy Cu on the  
 149 surface of these minerals with  $\Delta^{65}\text{Cu}_{\text{solid-solution}}$  values of up to 1‰ (Balistrieri et al., 2008;  
 150 Pokrovsky et al., 2008). Sorption to organic ligands shows varying fractionation with  
 151  $\Delta^{65}\text{Cu}_{\text{solid-solution}}$  values between -0.3 and 0.8‰ depending on type of organic ligand and pH  
 152 (Bigalke et al., 2010a; Navarette et al., 2011; Ryan et al., 2014). Lighter Cu isotopes are  
 153 preferentially adsorbed on clay mineral surfaces with  $\Delta^{65}\text{Cu}_{\text{adsorbed-solution}}$  values of -0.3‰ (Li

154 et al., 2015). Copper uptake into bacteria also causes various Cu isotope fractionations  
155 depending on organism and pH. Mainly the lighter Cu isotope is preferentially incorporated  
156 into the live bacteria cell ( $\Delta^{65}\text{Cu}_{\text{bacteria-solution}} = 0$  to  $-4.4$  ‰; Navarette et al., 2011; Pokrovsky  
157 et al., 2008; Zhu et al., 2002). In redox reactions, the reduced Cu species is enriched in lighter  
158 Cu isotopes (e.g.,  $\Delta^{65}\text{Cu}_{\text{solution-covellite}} = 3.06$ ‰; Ehrlich et al., 2004 and  $\Delta^{65}\text{Cu}_{\text{Cu}_2+\text{CuI}} = 4$  ‰;  
159 Zhu et al., 2002). Bigalke et al. (2010b, 2011, 2013) found episodically water-saturated soil to  
160 be enriched in heavy Cu isotopes, which they attributed to the loss of light Cu by leaching of  
161 reduced colloidal Cu forms. Ilina et al. (2013b) found no significant variations ( $\delta^{65}\text{Cu}$  values  
162 of 0.36 to 0.46 ‰) between differently filtered fractions ( $100\mu\text{m} - <1\text{kDa}$ ) of river water,  
163 which they attributed to similar bonding strength of Cu fulvic complexes of different mass.  
164 Vance et al. (2008) reported lighter  $\delta^{65}\text{Cu}$  values ( $-0.24$  to  $-1.02$ ‰) in the particulate fraction  
165 of rivers compared to the dissolved phase ( $0.42$  to  $0.94$ ‰) and explained this with strong  
166 bonding of dissolved Cu to organic complexes.

167 In this study, we investigate the isotope geochemistry of dissolved and colloidal Fe and Cu in  
168 a water-saturated carbonatic soil. Our goals are to better understand how a) Fe (oxyhydr)oxide  
169 dissolution and Fe colloid formation and b) Cu release and colloid formation in soils affect  
170 isotopic composition of soils and soil solutions. We furthermore will link our experimental  
171 results to field observations previously reported in the literature.

172

## 173 **2. Materials and methods**

174 A Calcaric Fluvisol (IUSS, 2014), with high calcium carbonate content ( $420\text{ g kg}^{-1}$ ) was sampled near  
175 the city of Aesch in Switzerland ( $47^{\circ}28'39''\text{ N} / 7^{\circ}36'21''\text{ E}$ ). The soil had a neutral pH (7.4), an  
176 organic carbon concentration of  $21.7\text{ g kg}^{-1}$ , a total Fe concentration of  $12.6\text{ g kg}^{-1}$  and a dithionite-  
177 extractable Fe concentration of  $7.87\text{ g kg}^{-1}$  (Abgottspon et al., 2015). More details about the soil are  
178 given in Table S1. The soil was located next to the river Birs on a floodplain with temporary flooding  
179 (Kayser et al., 2006). The sampling site was moderately contaminated with several trace metals (e.g.,



180 Cd, Cr, Cu, Pb, Table S1) by an adjacent non-ferrous metal smelter, causing elevated Cu  
181 concentrations of  $122 \text{ mg kg}^{-1}$  (Kusonwiriya Wong et al., 2016). About 10 kg of topsoil were sampled  
182 from the Ah horizon (0-0.15 m depth) in March 2011. The soil was air-dried and sieved to  $<2 \text{ mm}$ .  
183 The setup of the incubation experiment was similar to that of Weber et al. (2009b) and identical to that  
184 described in Abgottspon et al. (2015). In fact, samples were taken from exactly the same experiment  
185 described in Abgottspon et al. (2015). In summary, 1.7 L of artificial river water were filled in 3-L PE  
186 microcosms. The artificial river water (prepared from ultrapure water and clean salts) had a similar  
187 ionic strength and ionic composition as the river Birs next to the sampling site ( $0.92 \text{ mmol L}^{-1} \text{ CaCl}_2$ ,  
188  $0.98 \text{ mmol L}^{-1} \text{ NaNO}_3$ ,  $0.84 \text{ mmol L}^{-1} \text{ MgSO}_4$ ), but did not contain DOC, trace metals, or suspended  
189 matter. Then, 1.7 kg of air-dry soil was slowly added to the microcosm and stirred to ensure  
190 homogenization and removal of air bubbles, resulting in complete water saturation. After  
191 homogenization, the soil material was allowed to settle and around 2 cm of stagnant water covered the  
192 soil (Figure S1). Three replicate microcosms and one blank (i.e. only artificial river water) were set up  
193 in a glovebox (GS Glovebox Plexiglas, GS Glovebox Systemtechnik, Malsch, Germany) that was  
194 purged with nitrogen (Figure S2). The soil solution was extracted with PE suction cups with a  $10\text{-}\mu\text{m}$   
195 nominal pore size (ecoTech GmbH, Bonn, Germany) manually located in the lower half of the  
196 microcosms with a syringe. On each sampling date, 50 mL of solution was extracted. Over the whole  
197 experiment, we extracted 700 mL of a total solution volume of 1700 mL. Nevertheless, in the lower  
198 part of the microcosm the soil was always water-saturated (and the suction cup at this depth always  
199 flooded). Our extraction of solution might have simulated slow drainage as is also observed in natural  
200 soils. We realized mass balance calculations to check how repeated sampling of the microcosms may  
201 affect Fe and Cu concentrations and  $\delta^{56}\text{Fe}$  and  $\delta^{65}\text{Cu}$  values. Over the whole experiment we extracted  
202 0.06% and 0.18% of the total Fe and Cu in the system, respectively. Taking the isotopic composition  
203 into account, the isotopic change of the total pool related to the sampling is insignificant ( $-0.001\text{‰}$  and  
204  $0.002\text{‰}$ ) for  $\delta^{56}\text{Fe}$  and  $\delta^{65}\text{Cu}$ , respectively. These effects are much smaller than our analytical  
205 precision. Therefore, we conclude that the sampling will not significantly affect the isotopic  
206 composition of the total metal pools. The Eh and pH values were analysed directly after sampling in  
207 the glovebox. To rule out that the initial mobilization of colloids is an artifact caused by manually

208 sampling of the soil solution, Al concentrations were analyzed and found to be consistently low (on  
209 average  $0.5 \mu\text{mol L}^{-1}$ ). As Al is a major constituent of the soil (2.6 wt. %), low Al concentrations  
210 render the artificial release of soil colloids (e.g., clay minerals, Al hydroxides) unlikely. The solution  
211 is supposed to contain the total (colloidal + dissolved) element concentration ( $<10 \mu\text{m}$ ). To determine  
212 dissolved element concentrations, samples were manually filtered to  $<0.02 \mu\text{m}$  using syringe filters  
213 (Anotop 25 Plus, Whatman, Bottingen, Switzerland) in the glove box directly after sampling. The Fe  
214 and Cu concentrations were analyzed by ICP-MS (7700x, Agilent, Santa Clara, California) using In  
215 and Rh as internal standards. The difference between total and dissolved elemental concentrations was  
216 assumed to be the colloidal fraction ( $0.02\text{-}10 \mu\text{m}$ ). All unfiltered and filtered solutions were acidified  
217 with suprapur  $\text{HNO}_3$  (ROTH, Karlsruhe, Germany) and stored at  $-20^\circ\text{C}$  prior to isotope analysis. The  
218  $\text{Fe}^{2+}$  concentration in the unfiltered solution was determined photometrically (Spectroquant Pharo 100,  
219 MERCK). Each 5-mL aliquot of unfiltered soil solution was treated with  $50 \mu\text{L}$  of  $\text{H}_2\text{SO}_4$  (25%, p.a.),  
220  $0.5 \text{ mL}$  of ammonium-acetate-acetic acid ( $400 \text{ g L}^{-1}$  ammonium acetate, 50% acetic acid) and  $0.2 \text{ mL}$   
221 of phenanthroline solution ( $5 \text{ g L}^{-1}$  1,10-phenanthrolinechlorid) and made up to  $10 \text{ mL}$  with Millipore  
222 water in the glovebox. Samples were measured after 15 minutes at the wavelength of  $510 \text{ nm}$ . A  
223 detailed evolution of  $E_h$ , pH,  $\text{SO}_4^{2-}$ ,  $\text{S}^{2-}$ ,  $\text{Fe}^{2+}$  C and metal concentrations in 2-4 day resolution is given  
224 in Tables S2 & S3 and Abgottspon et al. (2015).

225 For total digestion of the bulk soil sample, approximately  $70 \text{ mg}$  of soil was digested  
226 in  $\text{HNO}_3$ , HF and  $\text{H}_2\text{O}_2$  (3:2:1) in PFA beakers (Savillex, MN, USA) at least  $24 \text{ h}$  on a hot  
227 plate at  $120^\circ\text{C}$ . The digested samples were evaporated until dryness at  $70^\circ\text{C}$ . Soil solution  
228 samples were evaporated in PFA vials (Savillex, MN, USA). The dried residues were digested  
229 using a mixture of concentrated  $\text{HNO}_3$  and  $\text{H}_2\text{O}_2$  (ratio 1:1) at  $120^\circ\text{C}$  for at least 3-4 hours and  
230 evaporated to dryness. The dried soil and solution samples were subsequently re-fluxed in  
231 HCl and  $\text{HNO}_3$  (3:1) at least  $3 \text{ h}$  at  $120^\circ\text{C}$  and finally dissolved in  $7 \text{ mol L}^{-1}$  HCl + 0.001%  
232  $\text{H}_2\text{O}_2$  for ion exchange separation. The total ( $<10\mu\text{m}$ ) and dissolved ( $<0.02\mu\text{m}$ ) fractions were  
233 analyzed in samples from Days 0, 2, 4, 6, 8, 11, 13, 16, 19, 22, 26, 30, 34 and 39. After 8 days  
234 of incubation, the dissolved Cu concentrations ( $<0.02\mu\text{m}$ ) were too low to get the required

235 300 ng of Cu for the isotope analysis. Thus, samples from the dissolved fraction of days 8, 10,  
236 13, and 16 were combined to a single composite sample and another composite sample was  
237 prepared from days 19, 22, 26, 30, 34 and 39. In these composite samples of the dissolved  
238 fraction, we also measured stable Fe isotope ratios after purification (see below). In addition,  
239 for Fe isotope measurement samples from the three replicates microcosms were combined to a  
240 composite sample of days 4 and 6, respectively, for both total (<10 $\mu$ m) and dissolved  
241 (<0.02 $\mu$ m) fractions because of too low mass (< 7 $\mu$ g Fe) of Fe for isotopic analysis at the  
242 beginning of the experiment. Copper and Fe fractions were purified as described in Bigalke et  
243 al. (2013). For solution samples, a single-step ion-exchange approach was sufficient to  
244 separate Cu and Fe from other matrix elements, while bulk soil samples were purified twice  
245 for Cu isotope analysis. All Fe and Cu fractions yielded an average recovery of 100.6 $\pm$ 1.3%  
246 (mean $\pm$ SD) and 101.1 $\pm$ 2.1% (mean $\pm$ SD), respectively. All samples which did not yield a  
247 recovery of within 100  $\pm$  6% were discarded, and the purification process was repeated. The  
248 acids (HNO<sub>3</sub> and HCl) used in this study were purified by sub-boiling distillation in quartz  
249 stills or were purchased in suprapur quality. Other reagents were of suprapur quality. All  
250 solutions and dilute acids were prepared using 18 M $\Omega$  grade water (EMD Millipore, MA,  
251 USA). All sample preparations were performed in the clean chemistry laboratory at the  
252 Institute of Geology, University of Bern. The procedural blank was 3.1 $\pm$ 1.8 ng (n=3) for Fe  
253 and 2.3 $\pm$ 0.2 ng (n=3) for Cu, compared to at least 7000 and 300 ng of Fe and Cu, respectively,  
254 in the samples.

255 Iron and Cu isotope measurements were performed on a Thermo-Scientific Neptune  
256 Plus MC-ICP-MS at the Leibniz University Hannover, Germany. The Fe isotopic  
257 compositions were analyzed in the high mass resolution mode to resolve isobaric  
258 interferences of <sup>40</sup>Ar<sup>14</sup>N<sup>+</sup>, <sup>40</sup>Ar<sup>16</sup>O<sup>+</sup>, and <sup>40</sup>Ar<sup>16</sup>OH<sup>+</sup> on <sup>54</sup>Fe, <sup>56</sup>Fe, and <sup>57</sup>Fe (Weyer and  
259 Schwieters, 2003). Chromium (<sup>53</sup>Cr) was monitored to correct isobaric interferences of <sup>54</sup>Cr  
260 on <sup>54</sup>Fe. The standard-sample bracketing method was applied in combination with external

261 element doping for mass bias correction. For the latter, the 5 mg L<sup>-1</sup> Ni standard NIST 986  
262 (National Institute of Standards and Technology, Gaithersburg, MA, USA) was doped to all  
263 Fe standards (IRMM-014, Institute for Reference Materials and Measurements, Geel,  
264 Belgium) and samples for mass-bias correction (Oeser et al., 2014). The Fe isotope standard  
265 (IRMM-014) and the samples were diluted to yield a concentration of 7 mg L<sup>-1</sup> Fe with 2%  
266 HNO<sub>3</sub>. Every sample was at least analyzed twice and the mean  $\delta^{56}\text{Fe}$  value is given. The  $\delta^{56}\text{Fe}$   
267 values are reported relative to IRMM-014.

268 In a three-isotope plot,  $\delta^{56}\text{Fe}$  and  $\delta^{57}\text{Fe}$  values define a linear correlation  
269 (slope=1.4664, R<sup>2</sup>= 0.99, Figure S3) following the theoretical mass-dependent fractionation  
270 line ( $\delta^{57}\text{Fe} \sim 1.5 \times \delta^{56}\text{Fe}$ ) which indicates the absence of isobaric interferences (Malinovsky et  
271 al., 2003). The accuracy and precision of the Fe isotope analysis was monitored using an Fe-  
272 free matrix sample (BCR-2, Basalt Columbia River 2, USGS, Reston, VA, USA) that was  
273 doped with Fe of known isotopic composition, and an in-house standard (an Fe salt from ETH  
274 Zurich, Switzerland). The Fe-free matrix samples were prepared from the matrix fraction  
275 derived from the purification of the original samples which were doped with the Fe standard  
276 (Merck Certipur<sup>®</sup>, Darmstadt, Germany) and processed in the same way as the samples. The  
277 average  $\delta^{56}\text{Fe}$  value of the matrix fraction was  $0.19 \pm 0.03\text{‰}$  (mean  $\pm 2\text{SD}$ , n=5) which is  
278 identical to the pure Merck Fe value ( $0.19 \pm 0.02\text{‰}$ , n=3). The USGS basalt BCR-2 yielded a  
279 mean  $\delta^{56}\text{Fe}$  value of  $0.07 \pm 0.02\text{‰}$  (2SD, n=4) which agrees well with previously published  
280 values ranging from  $0.05 \pm 0.02\text{‰}$  to  $0.11 \pm 0.03\text{‰}$  (Craddock and Dauphas, 2011; Liu et al.,  
281 2014a; Schuth et al., 2015; Weyer et al., 2005). The average  $\delta^{56}\text{Fe}$  value of our in-house  
282 standard was  $-0.69 \pm 0.02\text{‰}$  (mean  $\pm 2\text{SD}$ , n=6) and again in good agreement with previously  
283 published values varying from  $-0.71 \pm 0.18\text{‰}$  to  $-0.73 \pm 0.10\text{‰}$  (Fehr et al., 2008; Kiczka et al.,  
284 2011; Mansfeldt et al., 2012; Schuth et al., 2015).

285 For Cu isotope analysis, standard-sample bracketing was conducted in combination  
286 with external element doping for mass-bias correction. Again the Ni standard NIST 986 was

287 used for the mass-bias correction by analyzing  $^{62}\text{Ni}/^{60}\text{Ni}$  (for details see Lazarov and Horn,  
288 2015). The samples were diluted to yield a final concentration of  $300 \mu\text{g Cu L}^{-1}$  and  $1000 \mu\text{g}$   
289  $\text{Ni L}^{-1}$  and were measured with MC-ICP-MS in low resolution mode. Every sample was at  
290 least analyzed twice and the mean was reported. The  $\delta^{65}\text{Cu}$  values are reported relative to the  
291 Cu standard NIST SRM 976 after external mass-bias correction using the exponential law.  
292 The success of the Cu purification and the accuracy and precision of the Cu isotope analysis  
293 was verified by using Cu-free matrix samples that were subsequently doped with Cu of  
294 known isotopic composition, and a basalt reference material (BCR-2). The Cu-free matrix  
295 samples were prepared from the matrix fraction derived from the purification of the original  
296 samples and doped with the ERM<sup>®</sup>-AE633 Cu isotope standards (Institute for Reference  
297 Materials and Measurements, Geel, Belgium), which is isotopically indistinguishable from  
298 NIST 976 (Moeller et al., 2012). The doped matrices were treated and purified in the same  
299 manner as the original samples. The average  $\delta^{65}\text{Cu}$  value of the matrix samples was -  
300  $0.02 \pm 0.01\text{‰}$  (mean  $\pm$  2SD, n=3) which is identical to the value of ERM<sup>®</sup>-AE633 (-  
301  $0.01 \pm 0.05\text{‰}$ ; Moeller et al., 2012). The BCR-2 reference material yielded a mean of  
302  $\delta^{65}\text{Cu}_{\text{NIST976}} = 0.18 \pm 0.01\text{‰}$  (2SD, n=3), which is in good agreement with previously  
303 published data ranging from  $0.14 \pm 0.05\text{‰}$  to  $0.22 \pm 0.06\text{‰}$  (Moeller et al., 2012). The external  
304 reproducibility was evaluated with the in-house Cu standard (NBS C 125-2) yielding an  
305 average  $\delta^{65}\text{Cu}$  value of  $0.37 \pm 0.06\text{‰}$  (mean  $\pm$  2SD, n=10).

306 Most isotopic compositions from our experiments are reported as means of the  
307 triplicate microcosm incubations (Table 1). Therefore, error bars in the figures do not reflect  
308 measurement uncertainties but variations among the triplicate microcosms reflecting the  
309 natural soil heterogeneity. The isotopic composition of the metal colloidal fraction ( $\delta_{\text{coll}} =$   
310  $\delta^{56}\text{Fe}$  and  $\delta^{65}\text{Cu}$  values) was calculated from the difference in the isotopic composition of  
311 total and dissolved metal concentrations in solution based on isotopic mass balance, as show  
312 in Equation 1. To calculate colloidal concentrations and  $\delta$  values of the composite samples,

313 we averaged the values of the colloidal concentrations and the  $\delta$  values based on mass balance  
 314 approaches, as shown in Equation 2 with  $\delta_{ss}$  and  $C_{ss}$  being the delta value and the  
 315 concentration of the respective subsample. In Equation 1-5  $\delta_{tot}$ ,  $\delta_{diss}$  and  $\delta_{coll}$  are the  $\delta^{56}\text{Fe}$  or  
 316  $\delta^{65}\text{Cu}$  and  $c_{tot}$ ,  $c_{diss}$  and  $c_{coll}$  the concentrations of the total, dissolved and colloidal fraction,  
 317 while the prefix 2SD indicate the respective 2SD uncertainty.

$$318 \quad \delta_{coll} = \frac{(\delta_{tot} \times [c_{tot}]) - (\delta_{diss} \times [c_{diss}])}{[c_{tot}] - [c_{diss}]} \quad (1)$$

$$319 \quad \delta_{bulk} = \frac{\sum_{ss=1}^s \delta_{ss} \times [c_{ss}]}{\sum_{ss=1}^s [c_{ss}]} \quad (2)$$

320 The error of the  $\delta$  value of the colloidal fraction calculated according to the general formula  
 321 of error propagation (Taylor, 1997) as shown in Equation 3.

$$322 \quad 2SD\delta_{coll} = \sqrt{\left(\frac{\partial\delta_{coll}}{\partial\delta_{tot}} 2SD\delta_{tot}\right)^2 + \left(\frac{\partial\delta_{coll}}{\partial c_{tot}} 2SDc_{tot}\right)^2 + \left(\frac{\partial\delta_{coll}}{\partial\delta_{diss}} 2SD\delta_{diss}\right)^2 + \left(\frac{\partial\delta_{coll}}{\partial c_{diss}} 2SDc_{diss}\right)^2}$$

$$323 \quad (3)$$

324 If we apply Equation 3 to Equation 1, we get Equation 4, which allows calculating the  
 325 uncertainty of the  $\delta$  values of the colloidal fraction as displayed in Table 1.

$$326 \quad 2SD\delta_{coll} = \sqrt{\left(\frac{c_{tot}}{c_{tot} - c_{diss}} 2SD\delta_{tot}\right)^2 + \left(-\frac{c_{diss}(\delta_{tot} - \delta_{diss})}{(c_{tot} - c_{diss})^2} 2SDc_{tot}\right)^2 + \left(-\frac{c_{diss}}{c_{tot} - c_{diss}} 2SD\delta_{diss}\right)^2 + \left(\frac{(\delta_{tot} - \delta_{diss})c_{tot}}{(c_{diss} - c_{tot})^2} 2SDc_{diss}\right)^2}$$

$$327 \quad (4)$$

328 Finally, the error of the  $\Delta_{dissolved-colloidal}$  values was calculated according to equation 5

$$329 \quad 2SD\Delta_{diss-coll} = \sqrt{(2SD\delta_{diss})^2 + (2SD\delta_{coll})^2} \quad (5)$$

330 Table 1 Concentration and isotopic compositions of Fe and Cu in bulk soil prior to flooding, total (<10 $\mu\text{m}$ ), dissolved (<0.02  $\mu\text{m}$ ) and (calculated)  
 331 colloidal (0.02-10  $\mu\text{m}$ ) fractions of soil solution during 40 days of experimental flooding.

Element	Bulk soil / day after flooding	Total					Dissolved					Colloidal			
		Conc. ( $\mu\text{mol L}^{-1}$ )	SD	$\delta$ value (‰)	2SD	n <sup>a</sup>	Conc. ( $\mu\text{mol L}^{-1}$ )	SD	$\delta$ value (‰)	2SD	n <sup>a</sup>	Conc. <sup>h</sup> ( $\mu\text{mol L}^{-1}$ )	SD <sup>e</sup>	$\delta$ value <sup>f</sup> (‰)	2SD <sup>g</sup>
Fe	Soil	226000 <sup>b</sup>	22920 <sup>b</sup>	0.04	0.01	3	-	-	-	-	-	-	-	-	-
	4	41.4	2.86	-1.09	0.01	1	9.89	3.95	-0.85	0.01	1	31.6	4.88	-1.16	0.04
	6	116	0.24	-0.77	0.01	1	10.99	2.70	-1.56	0.01	1	105	2.71	-0.68	0.03
	8	181	7.36	-0.80	0.05	3	119 <sup>c</sup>	19.8	-1.86 <sup>b</sup>	0.03	1	197 <sup>c</sup>	29.4	0.03	0.26
	16	452	25.6	-0.64	0.04	3									
	39	673	9.17	-0.44	0.03	3	394 <sup>d</sup>	24.3	-0.98 <sup>c</sup>	0.01	1	203 <sup>d</sup>	25.9	0.32	0.14
Cu	Soil	1920 <sup>b</sup>	70 <sup>b</sup>	-0.08	0.04	3	-	-	-	-	-	-	-	-	-
	0	5.09	0.12	0.12	0.11	3	4.60	0.29	0.10	0.11	3	0.49	0.31	0.36	1.55
	2	23.0	1.59	-1.01	0.04	3	5.04	4.15	-0.37	0.04	3	18.0	4.45	-1.19	0.20
	4	20.4	2.30	-0.73	0.10	3	3.19	0.29	0.28	0.03	3	17.2	2.32	-0.92	0.12
	6	21.5	1.60	-0.50	0.13	3	0.53	0.23	1.04	0.03	1	21.0	1.62	-0.54	0.13
	8	14.4	1.46	-0.30	0.07	3									
	16	5.80	1.28	-0.14	0.08	3	0.07 <sup>c</sup>	0.03	0.68 <sup>c</sup>	0.04	1	10.1	1.37	-0.23	0.07
39	2.64	0.82	-0.41	0.07	3	0.07 <sup>d</sup>	0.02	0.38 <sup>d</sup>	0.03	1	2.59	0.82	-0.43	0.07	

332 <sup>a</sup>Number of replicate preparations and analysis, <sup>b</sup>Concentrations in the solid soil are in  $\mu\text{mol kg}^{-1}$ , <sup>c</sup>Bulked sample combined from days 8 to 16,

333 <sup>d</sup>Bulked sample combined from days 19 to 39, <sup>e</sup>Calculated by error propagation of standard deviation in total and dissolved concentration,

334 <sup>f</sup>Calculated by the difference between the  $\delta^{65}\text{Cu}$  values of total and dissolved Cu (Eq. 1), <sup>g</sup>Calculated by error propagation according to Eq. S1-S4,

335 <sup>h</sup>Calculated by subtracting the dissolved from the total concentrations.

### 336 3. Results

337 Iron was first mainly released in colloidal form, which changed after 19 days to  
338 predominantly dissolved Fe (Figure 1 and Table S3, Abgottspon et al., 2015). On day 39, only  
339 22% of the total Fe was still in colloidal form. The sum of total and dissolved Fe  
340 concentrations increased continuously until the end of the incubation, while the colloidal  
341 concentrations increased until day 16 and decreased afterwards (Figure 1). From day 2 on, the  
342 total Fe in solution was dominated by Fe<sup>2+</sup>, but the concentration of Fe<sup>2+</sup> started to deviate  
343 substantially from that of total Fe from day 13 on. After day 13, the Fe<sup>2+</sup> concentrations  
344 showed only a slight increase until day 22 and a decrease afterwards. From day 22 on, the  
345 dissolved Fe concentration was even higher than that of total Fe<sup>2+</sup> indicating that Fe<sup>3+</sup> was  
346 also present in the dissolved phase. On day 29, only 33% of the total Fe in solution was still  
347 Fe<sup>2+</sup>.

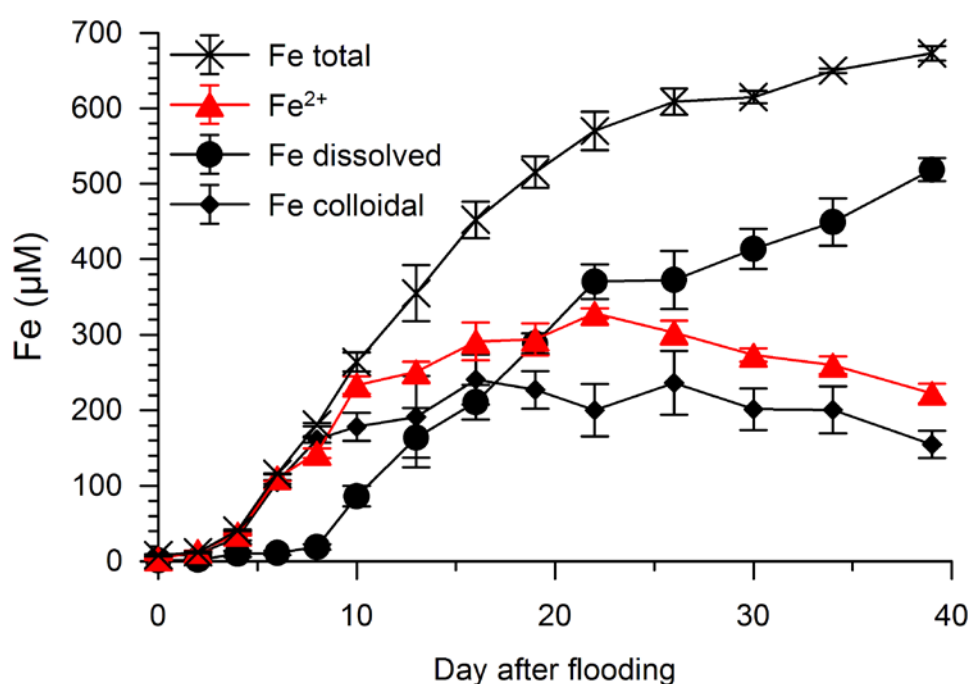
348 The Fe isotope composition of total Fe in solution started at negative  $\delta^{56}\text{Fe}$  values on day  
349 4 and afterwards increased slightly (Figure 2b). The  $\delta^{56}\text{Fe}$  value of total Fe in solution was  
350 correlated with the total Fe concentrations in solution (Figure 3). The  $\delta^{56}\text{Fe}$  value of the  
351 colloidal Fe sharply increased on day 6 and then gradually continued to increase until the end  
352 of incubation, and was parallel to the evolution of the  $\delta^{56}\text{Fe}$  values of the total Fe in solution  
353 (Figure 2b). The  $\delta^{56}\text{Fe}$  values of the dissolved fraction were higher than the colloidal fraction  
354 on day 4. Then,  $\delta^{56}\text{Fe}$  values of the dissolved fraction continuously decreased from day 4 to  
355 day 8-16. After 19-39 days, the  $\delta^{56}\text{Fe}$  values of dissolved Fe shifted to heavier values (Figure  
356 2b). The  $\Delta^{56}\text{Fe}_{\text{dissolved-colloidal}}$  value decreased from  $0.31 \pm 0.04\text{‰}$  on day 4 to  $-1.86 \pm 0.26\text{‰}$  on  
357 day 12 and thereafter increased slightly (Figure 4a). The  $\delta^{56}\text{Fe}$  value of the bulk soil was  
358  $0.04 \pm 0.01\text{‰}$  (n=3).

359 Copper was also mainly released in colloidal form but in contrast to Fe, Cu release  
360 peaked around day 6 and decreased thereafter (Figure 2c, Abgottspon et al., 2015). The  $\delta^{65}\text{Cu}$   
361 values of the total, dissolved and colloidal Cu fractions did not differ from each other and



362 were close to that of the bulk soil at the start of the experiment (day 0; Figure 2d). With  
 363 strongly increasing Cu concentrations in solution on day 2, the  $\delta^{65}\text{Cu}$  value of total Cu in  
 364 solution shifted to lower values and later on back towards initial values (Figure 2d). The  
 365  $\delta^{65}\text{Cu}$  value in both colloidal and dissolved Cu decreased markedly on day 2. After day 2, the  
 366  $\delta^{65}\text{Cu}$  value of the colloidal Cu increased until day 16; thereafter the  $\delta^{65}\text{Cu}$  value decreased  
 367 slightly until the end of the experiment. The  $\delta^{65}\text{Cu}$  value of the dissolved fraction increased as  
 368 well until day 6, but then, decreased towards the end of the experiment. The  $\Delta^{65}\text{Cu}_{\text{dissolved-}}$   
 369  $\text{colloidal}$  value changed from a maximum of  $1.58 \pm 0.13\text{‰}$  on day 6 back to  $0.81 \pm 0.03\text{‰}$  at the  
 370 end of the experiment (Figure 4b). The  $\Delta^{65}\text{Cu}_{\text{dissolved-colloidal}}$  from day 0 of the experiment could  
 371 not be used because of the high uncertainty of the  $\delta^{65}\text{Cu}_{\text{coll}}$  value at this day (Tab. 1). The  
 372  $\delta^{65}\text{Cu}$  value of the bulk soil was  $-0.08 \pm 0.04\text{‰}$  ( $n=3$ ).

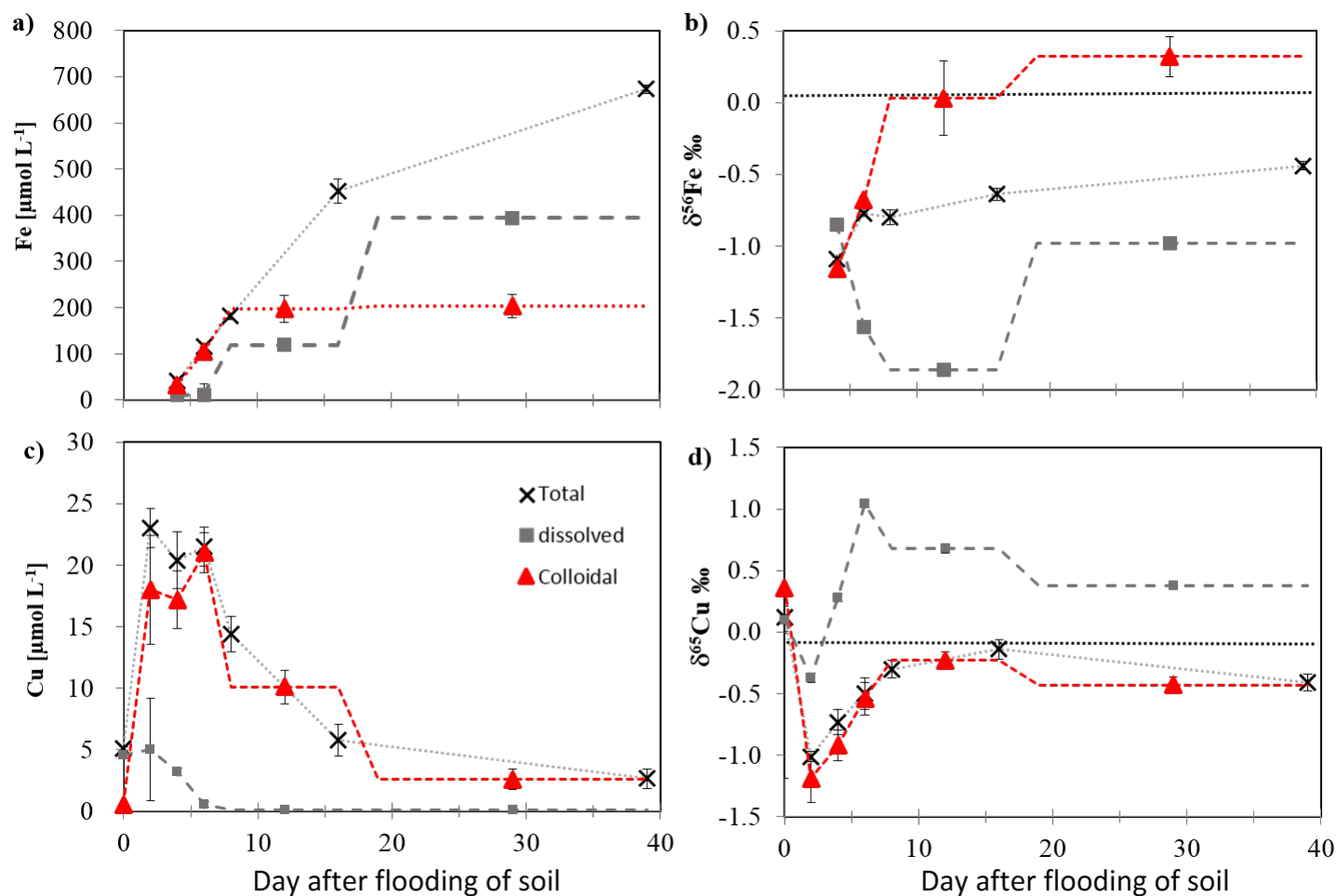
373



374

375 Figure 1 Fe concentrations in total, dissolved and colloidal Fe during the experiment. Total, dissolved  
 376 and colloidal Fe concentrations were taken from Abgottspon et al. (2015). The  $\text{Fe}^{2+}$  concentrations  
 377 were specifically measured for this study in unfiltered samples and therefore represent total  $\text{Fe}^{2+}$  in  
 378 solution including dissolved and colloidal fractions.

379



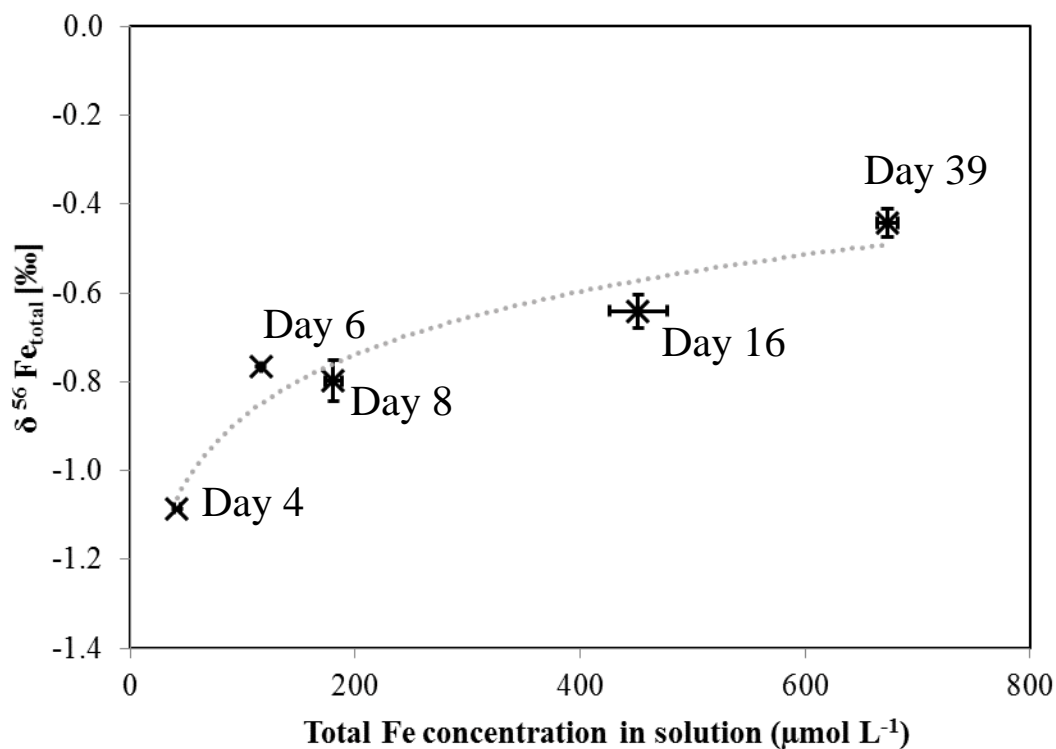
380

381 Figure 2 Temporal variation in a) Fe concentration, b) Fe isotope compositions, c) Cu concentration, and d) Cu isotope compositions during 40 days of flooding.  
 382 Connection lines of the single data points are displayed to guide the eye. Plateau-like connection lines for Fe and Cu are displayed because of the pooling of the  
 383 samples from different days. So every step corresponds to one sample. In Panels b and d, the dashed horizontal lines show the  $\delta^{56}\text{Fe}$  ( $0.04 \pm 0.01\text{‰}$ ) and  $\delta^{65}\text{Cu}$  ( $-$   
 384  $0.08 \pm 0.04\text{‰}$ ) values of the bulk soil, respectively. Vertical bars in Panels a and c indicate SD of concentrations from triplicate microcosm experiments. Vertical  
 385 bars in Panels b and d indicate 2SD for isotope values. Error bars are smaller than the symbols unless visible. Please note the different scales.

## 386 4. Discussion

### 387 4.1. Fe isotopes

388 After the flooding of soils, oxygen is consumed and electrons are released by  
389 microorganisms gaining energy from oxidation of organic matter. When the dissolved oxygen is  
390 used up, the electrons are transferred to alternative electron acceptors (Lovley, 1991). Iron  
391 (oxyhydr)oxides become the dominant alternative electron acceptors in flooded soils at  
392 sufficiently low  $E_h$  ( $< 150$  mV). In our experiment, the  $E_h$  value decreased quickly, reached 90  
393 mV four days after flooding and stabilized around 0 mV afterwards (Abgottspon et al., 2015).  
394 This decrease potentially triggers DIR, which is reflected in the temporal course of the total Fe  
395 concentration and  $\delta^{56}\text{Fe}$  value in solution in our experiment (Figure 3) and agrees with findings  
396 of Crosby et al. (2007), Johnson et al. (2005), but also with abiotic reduction reactions  
397 (Wiederhold et al., 2006).



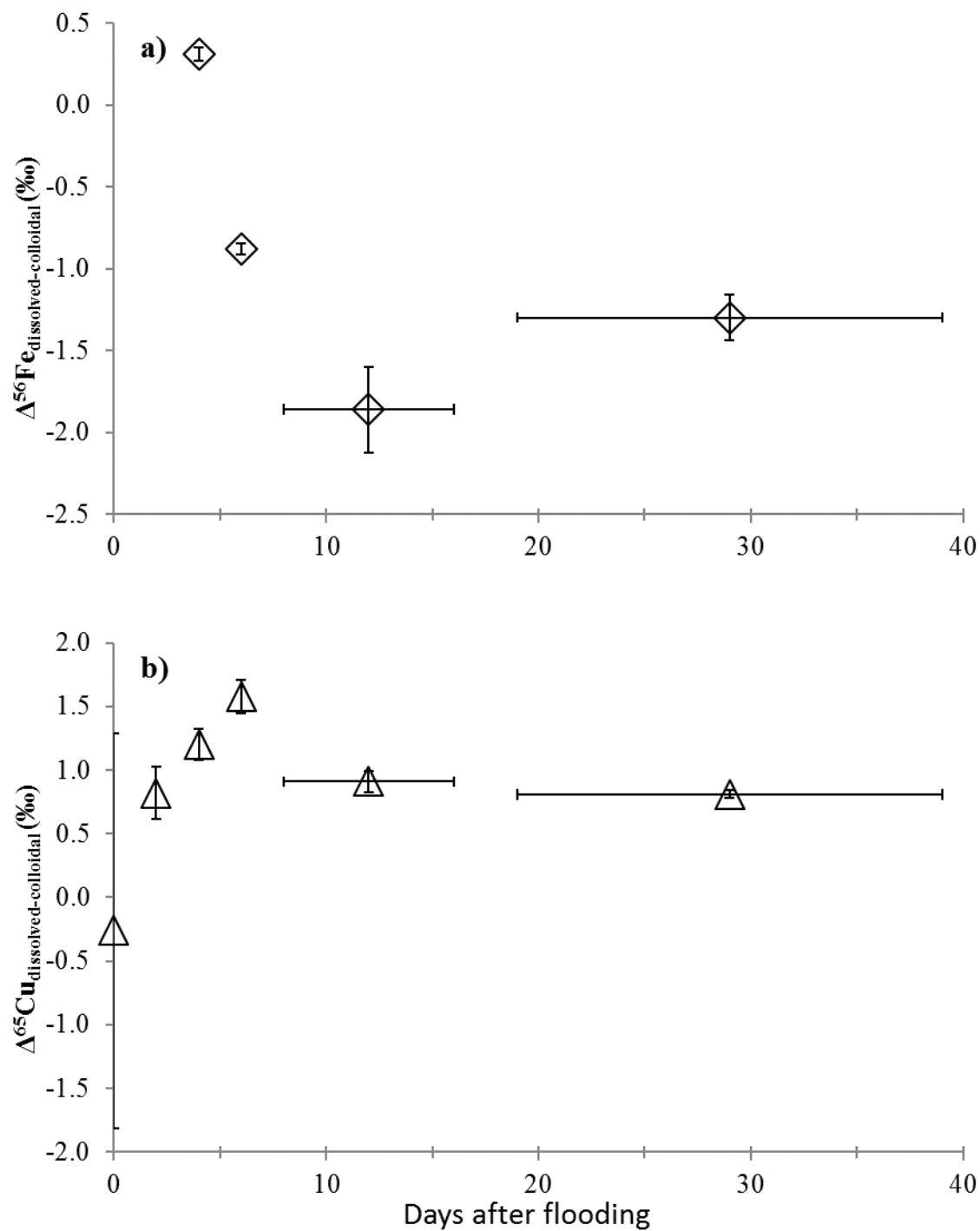
398

399 Figure 3  $\delta^{56}\text{Fe}$  values of total Fe in solution as a function of Fe concentrations during 40 days of flooding.  
400 Bars indicate SD of Fe concentrations from triplicate microcosm experiments and 2SD of Fe isotope  
401 compositions. Error bars are smaller than the symbols unless visible. The dotted line represents a  
402 logarithmic trend line.

403 From days 2-10, nearly all Fe in solution was  $\text{Fe}^{2+}$ , while the contribution of  $\text{Fe}^{2+}$  to total  
404 Fe in solution decreased after day 10 (Fig. 1), which is similar to the increase in  $\text{Fe}^{3+}$   
405 concentrations in solution reported by Schuth et al. (2015). After day 19, total dissolved Fe  
406 showed higher concentrations than  $\text{Fe}^{2+}$ , indicating the presence of  $\text{Fe}^{3+}$  even in the dissolved  
407 phase. Because the solubility of  $\text{Fe}^{3+}$  is very low, this finding may only be explained by small  
408  $\text{Fe}^{3+}$  colloids, which passed through the  $0.02\ \mu\text{m}$  filter (and are therefore operationally defined as  
409 dissolved species), or by the complexation of  $\text{Fe}^{3+}$  by organic substances, forming soluble  
410 complexes. Another possible explanation might be an analytical complication in the  
411 determination of the  $\text{Fe}^{2+}$  concentrations. If the Fe colloids at the end of the experiment did not  
412 fully dissolve when the sulphuric acid was added, the  $\text{Fe}^{2+}$  concentration might have been  
413 underestimated.

414 The pooling of the samples from different days, which was necessary because of the low  
415 Fe concentrations at the beginning of the experiment and low dissolved Cu concentrations at the  
416 end of the experiment, limit the temporal information of the Fe data. Therefore, we focus the  
417 further discussion on the Fe colloid release under anoxic conditions in soils, a process for which  
418 no Fe isotope data have yet been reported. The colloidal release plays an important role in our  
419 carbonatic floodplain soil. Up to 90% of the Fe was released in colloidal form on day 6 (Table  
420 S3; Abgottspon et al., 2015). Even in the later phase of the experiment (after day 10), colloidal  
421 Fe still accounted for 20-50% of total Fe in solution (Figure 2a). The lack of colloidal organic  
422 carbon and Al in solution (Abgottspon et al., 2015) indicate no co-mobilisation with OM or  
423 colloidal soil constituents like clay minerals, but rather Fe to be the main component of the

424 colloids (Table S2, S3). At the beginning of the experiment, total Fe and Fe<sup>2+</sup> concentrations in  
425 solution were identical, highlighting that colloids consisted of reduced Fe and are not Fe  
426 (oxyhydr)oxides released into soil solution (Figure 1). The colloidal Fe pool had low  $\delta^{56}\text{Fe}$   
427 values at the beginning, hence supporting the idea of precipitation from reduced light Fe<sup>2+</sup><sub>aq</sub>  
428 rather than mobilization by deflocculation of ferrihydrite, which should have a more positive  
429 value (span of the amorphous Fe oxide fraction in hydromorphic and oxic soils approx. -0.7 to  
430 0.4 ‰; Guelke et al., 2010; Schuth et al., 2015; Wiederhold et al., 2007a; Wiederhold et al.,  
431 2007b) compared to the colloidal fraction on day 4 of our experiment ( $-1.16 \pm 0.04$ ‰). Indeed, the  
432 initial  $\Delta^{56}\text{Fe}_{\text{dissolved-colloidal}}$  value was positive and fitted the values reported for siderite  
433 precipitation in laboratory experiments ( $\Delta^{56}\text{Fe}_{\text{dissolved-siderite}}$  values between 0.0 and 0.9‰;  
434 Johnson et al., 2005; Wiesli et al., 2004) and similar to that of lake sediments with supposed  
435 siderite precipitation (Figure 4a, Teutsch et al., 2009). However, when looking at the proposed  
436  $\Delta^{56}\text{Fe}_{\text{dissolved-colloidal}}$  value, it has to be kept in mind that isotopic fractionation of Fe in solution can  
437 also be influenced by interactions with the solid Fe pool in soil. Dissolved Fe<sup>2+</sup> released by DIR  
438 might be sorbed to Fe (oxyhydr)oxide surfaces in soil and undergo electron transfer-atom  
439 exchange reactions, a process which influences the  $\delta^{56}\text{Fe}$  value in solution (Crosby et al., 2005;  
440 2007) and might also affect the  $\Delta^{56}\text{Fe}_{\text{dissolved-colloidal}}$  values.



441

442

443 Figure 4 Temporal variations in apparent isotopic fractionation between dissolved and colloidal a) Fe and  
 444 b) Cu. Horizontal error bars represent the time interval of the pooled samples. Vertical error bars  
 445 represent the 2SD calculated according to Equations 3-5.

446

447 Abgottspon et al. (2015), suggested siderite precipitation from day 8 on in the same  
448 experiment. However, there is a time gap between the positive  $\Delta^{56}\text{Fe}_{\text{dissolved-colloidal}}$  on day 4 and  
449 the proposed siderite precipitation. Furthermore, a confirmation of siderite formation or the  
450 precipitation of other minerals by XRD analysis was not possible because of the low mass of  
451 colloidal material on the filters and interference of the filter material (an alumina-based  
452 membrane).

453 Siderite can be formed by the reaction between  $\text{Fe}^{2+}$  produced by DIR and  $\text{HCO}_3^-$  produced by  
454 microbial oxidation of organic matter (Mansfeldt et al., 2012; Wiesli et al., 2004). In our soil,  
455 high concentrations of inorganic carbon in solution ( $10 \text{ mmol L}^{-1}$  on day 4, Abgottspon et al.,  
456 2015) might cause a precipitation of  $\text{FeCO}_3$  directly from dissolved  $\text{Fe}^{2+}$  and  $\text{HCO}_3^-$  in solution  
457 at the beginning of the experiment. This is in agreement with the presence of colloidal inorganic  
458 carbon between days 2-19 of the experiment (Abgottspon et al., 2015), a dominance of colloidal  
459 Fe and the fact that total Fe in solution was  $\text{Fe}^{2+}$  until day 10 of the experiment. Therefore, even  
460 if we have no spectroscopic proof for siderite formation, we consider siderite to be the most  
461 likely Fe mineral in colloidal Fe based on solution chemistry, isotope results and previous  
462 findings (Abgottspon et al., 2015; Du Laing et al., 2009; Mansfeldt et al., 2012; Wiesli et al.,  
463 2004). However, already on day 6, the  $\Delta^{56}\text{Fe}_{\text{dissolved-colloidal}}$  changed, which may be explained by  
464 the following scenarios:

465 1. **Changing colloid mineralogy.** Mineralogy of the colloids may change with time,  
466 depending on changing solution chemistry and microbial community (Fulda et al., 2013; Roh  
467 et al., 2003). As an example, siderite and magnetite are formed in similar environments  
468 depending on the composition of the surrounding air and dissolved gases, the presence or  
469 absence of the carbonic acid-hydrogencarbonate buffer, pH, incubation temperature, type of

470 substrate, incubation time, and bacteria species (Roh et al., 2003). Sulfate reduction might  
471 induce formation of Fe sulfides (Borch et al., 2010). The change from  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$  in the soil  
472 solution and thus changing proportions of  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  in the dissolved and colloidal phases  
473 might explain the variations in the  $\Delta^{56}\text{Fe}_{\text{dissolved-colloidal}}$  values. Furthermore, the big gap  
474 between total Fe and total  $\text{Fe}^{2+}$  indicate that colloids change from a  $\text{Fe}^{2+}$  dominated mineral to  
475 a mineral containing also  $\text{Fe}^{3+}$ . Observed  $\Delta^{56}\text{Fe}_{\text{dissolved-colloidal}}$  values are consistent with those  
476 expected for a number of Fe minerals like e.g., mackinawite or magnetite (Friedrich et al.,  
477 2014; Guilbaud et al., 2011; Johnson et al., 2005; Wu et al., 2011).

478 **2. Sorption and electron transfer-atom exchange reactions.** The shift to lower  
479  $\Delta^{56}\text{Fe}_{\text{dissolved-colloidal}}$  values can also be caused by sorption and possibly electron transfer-atom  
480 exchange reactions at the surface of the colloids or soil components (Crosby et al., 2005;  
481 2007, Liu et al., 2015). The divalent dissolved Fe can be sorbed on these surfaces and electron  
482 transfer-atom exchange reactions may occur, which change isotopic signatures of Fe in  
483 solution (e.g., Friedrich et al., 2014). Sorption and electron transfer-atom exchange reactions  
484 on colloid surfaces would increase  $\delta^{56}\text{Fe}$  values in colloids and decrease  $\delta^{56}\text{Fe}$  values in  
485 solution, which is consistent with our results until day 16. However, also sorption to Fe  
486 oxy(hydr)oxide surfaces in the soil and electron transfer-atom exchange reactions, might  
487 explain a significant part of the observed  $\delta^{56}\text{Fe}$  variation in the solution (Reddy et al., 2015).  
488 After 16 days of flooding, the shift of dissolved Fe to higher  $\delta^{56}\text{Fe}$  values can be attributed to  
489 an isotopic pool size effect caused by proceeding DIR and the growth of the dissolved relative  
490 to the colloidal Fe fraction (Figure 2b).



491 However, the changing  $\delta^{56}\text{Fe}$  values together with the increasing or constant colloid  
492 concentration, indicated that new Fe colloids were formed throughout, while the concentrations  
493 of Cu colloids steadily decreased probably because of aggregation and sedimentation.

494 In a recent experiment, the same soil was incubated under the same experimental conditions  
495 and changes in the partitioning of Fe into five operatively defined fractions (F1–F5;  $\text{NH}_4\text{NO}_3$ -  
496 extractable, NaOAc-extractable,  $\text{NH}_4\text{Ox}$ -extractable, hot  $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ -extractable and residual  
497 fractions, respectively) were investigated (Abgottspon et al., 2015). At the beginning of the  
498 experiment 81 % of the Fe in the soil was in the residual fraction which is supposed to consist  
499 mainly of silicate-bound Fe and crystalline Fe (oxyhydr)oxides, the second important fraction  
500 was the oxalate-extractable fraction (15 %, amorphous Fe oxides), while the other fractions had  
501 minor importance (< 3 %). Over the time of the incubation, Fe concentrations in F1-F4  
502 increased, indicating a substantial redistribution of Fe, which is only partly mirrored in the  
503 solution chemistry.

504 A variation in solid phase  $\delta^{56}\text{Fe}$  values of up to 0.93‰ was reported for soils which are  
505 seasonally or permanently water-saturated (Fekiacova et al., 2013; Liu et al., 2014b; Mansfeldt et  
506 al., 2012; Schuth et al., 2015; Wiederhold et al., 2007a). This can be attributed to the  
507 mobilization of reduced isotopically light Fe, which might be leached, leaving the soil enriched  
508 in heavier Fe isotopes (Schuth and Mansfeldt, 2016). The fractionation follows an apparent  
509 Rayleigh-type behavior with smaller fractionation factors in soils compared to experimentally  
510 found values, because of additional processes (e.g., organic complexation and reprecipitation)  
511 occurring in the complex soil system (Garnier et al. 2017, Thompson et al., 2007). In contrast,  
512 most soils that developed under oxic conditions only display a small variation in bulk solid phase  
513  $\delta^{56}\text{Fe}$  values of 0.08‰ (Podzols show variations in  $\delta^{56}\text{Fe}$  values of up to 0.6‰; Poitrasson et al.,

514 2008; Wiederhold et al., 2007b). In agreement with these findings, we observed a release of light  
515 Fe into the soil solution ranging from -1.1‰ to -0.4‰ (Figure 2b). The evolution of the  $\delta^{56}\text{Fe}$   
516 values in total Fe during our experiment showed a time dependency with low  $\delta^{56}\text{Fe}$  values  
517 shortly after flooding which successively approached bulk soil values with increasing time of  
518 flooding.

519 These findings agree with the interpretation of the variation in  $\delta^{56}\text{Fe}$  values of total Fe in the  
520 water of the Rio Negro River, where a substantial temporal variation was observed (dos Santos  
521 Pinheiro et al., 2014). In Rio Negro River water, the lowest  $\delta^{56}\text{Fe}$  values were coupled to strong  
522 rain events, which cause a reductive mobilization of Fe from the soils in the river catchment  
523 (Bergquist and Boyle, 2006). Hence, colloidal Fe release in certain anoxic soils significantly  
524 affects the temporal isotopic variability of the colloidal fraction. For a detailed understanding of  
525 the processes causing the isotopic changes in the colloid fraction, further investigation of the  
526 associated processes and mineralogy will be needed which is beyond the scope of this study.

527 Overall, the agreement of the results from our microcosm experiment and field observations  
528 confirms that such experiments can be used for future research into Fe isotopic behavior in  
529 anoxic soils. A big advantage of the microcosms is that soil solution samples can be extracted  
530 under controlled oxygen-free conditions and allow e.g., for the separation of colloids from  
531 dissolved Fe in anoxic samples or photometric  $\text{Fe}^{2+}$  analysis. This separation is hardly possible  
532 under oxic field conditions as reduced  $\text{Fe}^{2+}$  will almost immediately precipitate as Fe  
533 (oxyhydr)oxides when coming in contact with the atmosphere. In spite of the limitations of our  
534 study because we had to pool bulk samples of several dates and the lack of direct  
535 characterization of the mineral phases, our results indicate that the Fe release in colloidal form

536 contributes substantially to total Fe concentrations and  $\delta^{56}\text{Fe}$  values in solution and thus to Fe  
537 mobility in our study soil.

538

#### 539 **4.2. Cu isotopes**

540 The initial apparent isotopic fractionation between Cu in solution (total, dissolved and  
541 colloidal) and in bulk soil was small (Figure 2d). With increasing time after flooding, the  
542 fractionation between bulk soil and solution and between dissolved and colloidal Cu species in  
543 solution increased, with total and colloidal fraction becoming lighter and the dissolved fraction  
544 becoming heavier. Relating the Cu isotope ratios to a similar experiment in which Cu speciation  
545 was investigated by XAS on a polluted soil from the River Mulde in Germany, offers a possible  
546 explanation for most of the observed isotope fractionations. The Cu concentration peak on day 2  
547 and the strong shift to negative  $\delta^{65}\text{Cu}$  values of total Cu in solution could be caused by Cu-  
548 carrying bacteria cells which were dominating the colloidal phase in studies with other soils  
549 (Hofacker et al., 2013; Weber et al., 2009a). The simultaneously observed heavy Cu isotope ratio  
550 of the remaining Cu in solution would then be the consequence of previous preferential uptake of  
551  $^{63}\text{Cu}$  by the bacteria (Navarette et al., 2011; Pokrovsky et al., 2008), Cu reduction in the cell and  
552 the change to trigonal coordination (Weber et al., 2009a). The shift in  $\delta^{65}\text{Cu}$  values between  
553 dissolved and colloidal Cu was similar to previously reported Cu isotope fractionation that was  
554 linked to the uptake of Cu into bacteria with  $\Delta^{65}\text{Cu}_{(\text{solution-bacteria})}$  values between +1.0‰ and  
555 +4.4‰ (Navarette et al., 2011). The change of  $\delta^{65}\text{Cu}$  values in solution between days 2 and 6,  
556 while total Cu concentrations remained stable, can only be explained by the exchange of solution  
557 Cu with solid soil Cu pools. The shift to higher  $\delta^{65}\text{Cu}$  values from  $-1.01 \pm 0.04\text{‰}$  to  $-0.50 \pm 0.13\text{‰}$   
558 in total Cu in solution requires the removal of isotopically light Cu from solution and/or release

559 of heavy Cu into solution. In the experiments of Hofacker et al. (2013) and Weber et al. (2009a),  
560 bacteria released  $\text{Cu}^+$ , which disproportioned into  $\text{Cu}^{2+}$ , released into solution, and zero-valent  
561 Cu ( $\text{Cu}^0$ ) colloids, in this phase of the incubation. In solution,  $\text{Cu}^{2+}$  released from bacteria was  
562 partly reduced and precipitated together with the already present Cu in soil solution and  
563 exchangeable Cu from the solid soil as  $\text{Cu}_x\text{S}$  (Weber et al., 2009b). If this process also occurred  
564 in our experiment the  $\text{Cu}_x\text{S}$  would be a mixture of soil Cu and solution Cu and would probably  
565 carry a heavier isotopic signal than that of the Cu in the bacteria. After day 6 with the onset of  
566 sulfate reduction (Table S2, Abgottspon et al., 2015), Cu isotope composition and apparent  
567 fractionation between dissolved and colloidal Cu stabilized (average values of  $\delta^{65}\text{Cu} = -$   
568  $0.29 \pm 0.15\text{‰}$  and  $\Delta^{65}\text{Cu}_{\text{dissolved-colloidal}} = 0.87 \pm 0.09\text{‰}$ ) despite decreasing Cu concentrations in  
569 solution. These findings indicate that the further decrease of colloidal Cu was caused by a  
570 progressive aggregation and sedimentation of colloids, which does not cause a pronounced  
571 isotopic fractionation (Figure 4b). Even if the results from the soil investigated by Hofacker et al.  
572 (2013) and Weber et al. (2009a) cannot be directly transferred to our soil because of the acid soil  
573 used by these authors and our slightly alkaline soil, Abgottspon et al. (2015) showed that in our  
574 soil decreasing metal mobilization paralleled increasing sulfate reduction. However, the fact that  
575 we did not observe colloidal organic carbon at the beginning of the experiment (Table S2)  
576 disagrees with the findings of bacterial release causing the increase of colloidal Cu at the  
577 beginning of the experiment (Hofacker et al., 2013; Weber et al. 2009a). Assuming a C/Cu (w/w)  
578 ratio of about 4000 as described for methanogenic bacteria (Scherer et al., 1983), a bacterial  
579 release causing  $23 \mu\text{mol Cu L}^{-1}$  on day 2 should be clearly visible also in colloidal C (approx.  
580  $500 \text{ mmol L}^{-1}$ ). Even if the strong variation of Cu concentrations in bacteria is taken into

581 account, the colloidal C concentration should be clearly higher than the measured  $0.2 \text{ mmol L}^{-1}$   
582  $C_{\text{coll}}$  (Table S2), if bacteria caused the colloidal Cu concentration on day 2.

583 One might hypothesize that the reported changes of Cu redox states (Weber et al., 2009a)  
584 explain much of the observed  $\Delta^{65}\text{Cu}_{\text{dissolved-colloidal}}$  values in soil solution, which are  $0.82 \pm 0.20\text{‰}$   
585 on day 2, when Cu is supposed to occur as  $\text{Cu}^+$  and reach a maximum value of  $1.58 \pm 0.13\text{‰}$  on  
586 day 6 as a result of the presumed dominance of  $\text{Cu}^0$ . After sulfate reduction on days 6-8 (Table  
587 S1),  $\Delta^{65}\text{Cu}_{\text{dissolved-colloidal}}$  values changed back to  $0.81 \pm 0.03\text{‰}$ , in line with the assumption that  
588  $\text{Cu}^+$  was the dominant Cu species (Weber et al., 2009a; Figure 4b). However, it is not clear, if the  
589 same redox transformations as described by Weber et al. (2009a) occur in the soil used in our  
590 experiment. Generally, the isotopic fractionation associated with abiotic oxidative leaching of  
591 reduced Cu minerals (chalcocite, chalcopyrite, enargite) is in the range of  $\Delta^{65}\text{Cu}_{\text{aq-mineral}} = 0$  to  
592  $2.7\text{‰}$  in laboratory experiments and field surveys (Fernandez and Borrok, 2009; Kimball et al.,  
593 2008; Mathur et al., 2005) and agree well with theoretical estimations stating that Cu(I)  
594 complexes preferentially enrich  $^{63}\text{Cu}$  and their precipitation results in the enrichment of  $^{65}\text{Cu}$  in  
595 the fluid (Fuji et al., 2013). This pattern supports the interpretation that the observed  
596  $\Delta^{65}\text{Cu}_{\text{dissolved-colloidal}}$  values in our experiment are caused by precipitation of reduced colloidal Cu  
597 species. However, fractionation has been reported to be irrelevant or even in the opposite  
598 direction in the presence of bacteria (Kimball et al., 2008, Mathur et al., 2005). The latter has  
599 been explained with the accumulation of heavy isotopes on the bacteria and preferential  
600 precipitation of heavy Cu on bacteria surfaces. The biotic induced isotope fractionation is  
601 counteracting the isotope fractionation introduced by abiotic redox processes. Thus in a system  
602 with reducing conditions and bacteria the  $\delta^{65}\text{Cu}$  values in solution are determined by the balance  
603 of Cu released abiotically and Cu that interacts with the cells and biotic precipitates (Mathur et

604 al., 2005, Kimball et al., 2008). If we transfer the latter findings to our  $\Delta^{65}\text{Cu}_{\text{dissolved-colloidal}}$  values,  
605 this might indicate that abiotic reduction rather than bacterial Cu precipitation is governing  
606 colloid formation. However, the influence of microorganisms as well as other soil processes (e.g.  
607 organic complexation or reprecipitation as described by Thompson et al., 2007 for Fe), might  
608 decrease the apparent fractionation in our soil, compared to experimentally determined  
609 fractionation factors in less complex systems (e.g., Fernandez and Borrok, 2009; Zhu et al.,  
610 2002).

611 In a recent experiment (Kusonwiriya Wong et al., 2016), we determined the changes in Cu  
612 partitioning and the  $\delta^{65}\text{Cu}$  values in five fractions of a sequential extraction (F1–F5;  $\text{NH}_4\text{NO}_3$ -  
613 extractable, NaOAc-extractable,  $\text{NH}_4\text{Ox}$ -extractable, hot  $\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ -extractable and residual  
614 fractions, respectively) in the same soils that we used in this study. We found that in the dry soil,  
615  $\delta^{65}\text{Cu}$  values in F1–F4 followed the estimated bonding strengths of Cu in the respective  
616 fractions, indicating equilibrium distribution of Cu at the beginning of the experiment. After  
617 flooding, Cu concentrations decreased in F1–F3 and increased in F4–F5. Overall, 73% of the  
618 total Cu was redistributed among the five studied fractions. The strongest variations in  $\delta^{65}\text{Cu}$   
619 values occurred in F3 ( $0.09 \pm 0.07\text{‰}$  to  $1.43 \pm 0.13\text{‰}$ ) and F4 ( $-0.24 \pm 0.07\text{‰}$  to  $0.55 \pm 0.07\text{‰}$ ),  
620 while flooding had no or small effects on the  $\delta^{65}\text{Cu}$  values of F1, F2 and F5. So even if F1  
621 should be the Cu pool, which interacts most strongly with the solution, it did not mirror the shifts  
622 of the dissolved fractions in this experiment. The results from Kusonwiriya Wong et al. (2016)  
623 suggest a direct transfer of Cu from F3 to F4 because both concentration changes and changes in  
624  $\delta^{65}\text{Cu}$  values were balanced between the two fractions. The responses of Cu partitioning and  
625  $\delta^{65}\text{Cu}$  values to flooding are in line with the formation of  $\text{Cu}_x\text{S}$  or other reduced Cu species and  
626 the reduction of Cu associated with Fe (oxyhydr)oxides.

627           One factor which can only be traced with the used isotopic approach is the exchange of  
628 Cu in solution with solid soil Cu, which is most pronounced between days 2-6. On day 8, the  
629 total  $\delta^{65}\text{Cu}$  value of the soil solution showed good agreement with that of Fraction 4 of the  
630 sequential extraction of the solid soil by Kusonwiriawong et al. (2016) on day 7, which is  
631 supposed to represent reduced Cu phases. The agreement in these two  $\delta^{65}\text{Cu}$  values supports the  
632 assumption, that at this time colloidal Cu in solution and solid soil Cu are coupled. However, the  
633 Cu isotope signatures in Fraction 4 in the solid soil changed to positive values ( $0.55\pm 0.07\text{‰}$ ) on  
634 day 35, while dissolved Cu still showed negative  $\delta^{65}\text{Cu}$  values and did not change significantly  
635 between days 8 and 39. This suggests that after the initial peak Cu colloid concentration, colloid  
636 formation and exchange with the soil stopped, while aggregation in solution decreased the Cu  
637 colloid concentration.

638           Babcsanyi et al. (2014) and Bigalke et al. (2010b, 2011, 2013) investigated different oxic  
639 and anoxic soils and wetland systems and found that Cu isotope signatures were isotopically  
640 heavier in temporally flooded soils than in soils, which developed in an oxic environment. They  
641 concluded that isotopically light Cu was released from flooded wetlands. Furthermore, these  
642 authors suggested that leaching of reduced Cu colloids was responsible for the observed apparent  
643 fractionation. This assumption is confirmed by our findings, which demonstrate a strong release  
644 of light colloidal Cu shortly after flooding. Leaching of this Cu from the soil would cause the  
645 described shift to heavier  $\delta^{65}\text{Cu}$  values. The extent of the fractionation in real soils would depend  
646 on the progress of leaching. In our study soil, the lowest  $\delta^{65}\text{Cu}$  value for total Cu in solution  
647 occurred on day 2. Assuming a similar  $\delta^{65}\text{Cu}$  value ( $-1.0\text{‰}$ ) of the total Cu released into solution  
648 and leached over several redox cycles and a leaching of e.g., 10% of the initial Cu in bulk soil,  
649 the total Cu in bulk soil should be isotopically shifted from initially 0 to  $+0.1\text{‰}$ .

650 This mobilization of Cu has to be considered in the management of polluted sites, by  
651 preventing changes of redox conditions. Especially, floodplain soils, which show high metal  
652 concentrations in many cases, might directly leach the Cu to the associated river, and thus  
653 negatively affect the river ecosystem. Because the colloids are carrying up to 100% of Cu and  
654 associated trace metals in soil solution and will strongly affect transport behavior and toxicity  
655 (Griffitt et al., 2007; Ju-Nam and Lead, 2008), the extent and stability of colloids formed in  
656 carbonatic soils need to be considered in risk assessments.

657

## 658 5. Conclusions

- 659 • Total  $\delta^{56}\text{Fe}$  values in solution followed the pattern of DIR. The  $\Delta^{56}\text{Fe}_{\text{dissolved-colloidal}}$  values  
660 indicated that dissolved Fe precipitated directly to form colloids, which accounted for  
661 90% of total Fe at the beginning to 20% of total Fe at the end of the experiment. While  
662 siderite precipitation might be one reason for colloidal Fe formation, a change of  
663  $\Delta^{56}\text{Fe}_{\text{dissolved-colloidal}}$  values with time indicates either changes in the colloid mineralogy, or  
664 sorption and possible electron transfer-atom exchange reactions with colloidal Fe or Fe  
665 minerals in the soil solid phase. The results match previous findings from lake, river and  
666 soil environments and underline the significance of colloid formation from dissolved Fe  
667 in some anoxic soil environments. The strong changes in  $\Delta^{56}\text{Fe}_{\text{dissolved-colloidal}}$  values raise  
668 new questions about formation and changes of the Fe colloid composition under anoxic  
669 conditions.
- 670 • The  $\Delta^{65}\text{Cu}_{\text{dissolved-colloidal}}$  seemed to be mainly driven by Cu redox state. The  $\delta^{65}\text{Cu}$  values  
671 indicated a strong exchange of colloidal Cu with solid soil Cu and a decoupling of these  
672 two pools with beginning sulfate reduction and decreasing Cu concentrations in solution.



673 The comparison of the experimental results with previous findings from hydromorphic  
674 soils and wetlands indicates that Cu colloid formation might be an important factor  
675 driving Cu leaching in these environments. Therefore, Cu colloid formation should be  
676 accounted for in risk assessment of e.g., polluted floodplain soils and similar  
677 environments.

678 In this study, the first results of Fe and Cu isotopic composition of colloids forming in anoxic  
679 soil environments are presented. Both elements are redox sensitive and probably undergo  
680 reduction, but show distinctly different temporal concentration and isotopic patterns. Both  
681 elements have a considerable colloidal component in common. Colloidal Fe and Cu might cause  
682 colloidal co-mobilization of a line of other potential toxic elements in soils. Furthermore,  
683 colloidal properties need to be considered in toxicity and mobility assessments.

684

#### 685 **Acknowledgements**

686 We thank the group of Isotope Geology of the University of Berne, Klaus Mezger, Thomas  
687 Nägler, Igor Villa and Gabriela Balzer for access to the clean room and support. We thank the  
688 AE Michael Böttcher and the two reviewers Ryan Mathur and Jan Wiederhold for their  
689 constructive comments, which considerably improved the manuscript. We also thank the  
690 Agricultural Research Development Agency (Public Organization), ARDA, Thailand, for  
691 funding Charirat Kusonwiriawong.

692

#### 693 **Supporting Material**

694 Supporting material for this manuscript is available, including soil characterization, solution  
695 chemistry data and figures illustrating the experimental setup and a three isotope plot for the Fe  
696 isotope analysis.

697

698

699 **References**

- 700 Abgottspon, F., Bigalke, M., Wilcke, W., 2015. Mobilization of trace elements in a carbonatic  
701 soil after experimental flooding. *Geoderma* 259-260, 156-163.
- 702 Babcsanyi, I., Imfeld, G., Granet, M., Chabaux, F., 2014. Copper stable isotopes to trace copper  
703 behavior in wetland systems. *Environ. Sci. Technol.* 48(10), 5520-5529.
- 704 Balistrieri, L.S., Borrok, D.M., Wanty, R.B., Ridley, W.I., 2008. Fractionation of Cu and Zn  
705 isotopes during adsorption onto amorphous Fe(III) oxyhydroxide: Experimental mixing  
706 of acid rock drainage and ambient river water. *Geochim. Cosmochim. Acta* 72(2), 311-  
707 328.
- 708 Bergquist, B.A., Boyle, E.A., 2006. Iron isotopes in the Amazon River system: Weathering and  
709 transport signatures. *Earth Planet. Sci. Lett.* 248(1-2), 54-68.
- 710 Bigalke, M., Kersten, M., Weyer, S., Wilcke, W., 2013. Isotopes trace biogeochemistry and  
711 sources of Cu and Zn in an intertidal soil. *Soil Sci. Soc. Am. J.*  
712 doi:10.2136/sssaj2012.0225.
- 713 Bigalke, M., Weyer, S., Wilcke, W., 2010a. Copper isotope fractionation during complexation  
714 with insolubilized humic acid. *Environ. Sci. Technol.* 44(14), 5496-5502.
- 715 Bigalke, M., Weyer, S., Wilcke, W., 2010b. Stable Copper Isotopes: A novel tool to trace copper  
716 behavior in hydromorphic soils. *Soil Sci. Soc. Am. J.* 74(1), 60-73.
- 717 Bigalke, M., Weyer, S., Wilcke, W., 2011. Stable Cu isotope fractionation in soils during oxic  
718 weathering and podzolization. *Geochim. Cosmochim. Acta* 75(11), 3119-3134.
- 719 Borch, T., Kretzschmar, R., Kappler, A., Van Cappellen, P., Ginder-Vogel, M., Voegelin, A.,  
720 Campbell, K., 2010. Biogeochemical redox processes and their impact on contaminant  
721 dynamics. *Environ. Sci. Technol.* 44(1), 15-23.
- 722 Brantley, S.L., Liermann, L., Bullen, T.D., 2001. Fractionation of Fe isotopes by soil microbes  
723 and organic acids. *Geology* 29(6), 535-538.
- 724 Burton, E.D., Bush, R.T., Sullivan, L.A., Johnston, S.G., Hocking, R.K., 2008. Mobility of  
725 arsenic and selected metals during re-flooding of iron- and organic-rich acid-sulfate soil.  
726 *Chem. Geol.* 253(1-2), 64-73.
- 727 Butler, I.B., Archer, C., Vance, D., Oldroyd, A., Rickard, D., 2005. Fe isotope fractionation on  
728 FeS formation in ambient aqueous solution. *Earth and Planetary Science Letters*, 236(1-  
729 2): 430-442.

- 730 Contin, M., Mondini, C., Leita, L., De Nobili, M., 2007. Enhanced soil toxic metal fixation in  
731 iron (hydr)oxides by redox cycles. *Geoderma* 140(1-2), 164-175.
- 732 Craddock, P.R., Dauphas, N., 2011. Iron Isotopic compositions of geological reference materials  
733 and chondrites. *Geostand. Geoanal. Res.* 35(1), 101-123.
- 734 Crosby, H.A., Roden, E.E., Johnson, C.M., Beard, B.L., 2007. The mechanisms of iron isotope  
735 fractionation produced during dissimilatory Fe(III) reduction by *Shewanella putrefaciens*  
736 and *Geobacter sulfurreducens*. *Geobiology* 5(2), 169-189.
- 737 Dideriksen, K., Baker, J.A., Stipp, S.L.S., 2008. Equilibrium Fe isotope fractionation between  
738 inorganic aqueous Fe(III) and the siderophore complex, Fe(III)-desferrioxamine B. *Earth*  
739 *Planet. Sci. Lett.* 269(1-2), 280-290.
- 740 Dos Santos Pinheiro, G.M.D., Poitrasson, F., Sondag, F., Cochonneau, G., Vieira, L.C., 2014.  
741 Contrasting iron isotopic compositions in river suspended particulate matter: the Negro  
742 and the Amazon annual river cycles. *Earth Planet. Sci. Lett.* 394, 168-178.
- 743 Du Laing, G., Rinklebe, J., Vandecasteele, B., Meers, E., Tack, F.M.G., 2009. Trace metal  
744 behaviour in estuarine and riverine floodplain soils and sediments: A review. *Sci. Total*  
745 *Environ.* 407(13), 3972-3985.
- 746 Du Laing, G., Vanthuyne, D.R.J., Vandecasteele, B., Tack, F.M.G., Verloo, M.G., 2007.  
747 Influence of hydrological regime on pore water metal concentrations in a contaminated  
748 sediment-derived soil. *Environ. Pollut.* 147(3), 615-625.
- 749 Ehrlich, S., Butler, I., Halicz, L., Rickard, D., Oldroyd, A., Matthews, A., 2004. Experimental  
750 study of the copper isotope fractionation between aqueous Cu(II) and covellite, CuS.  
751 *Chem. Geol.* 209(3-4), 259-269.
- 752 Fehr, M.A., Andersson, P.S., Halenius, U., Morth, C.M., 2008. Iron isotope variations in  
753 Holocene sediments of the Gotland Deep, Baltic Sea. *Geochim. Cosmochim. Acta*  
754 72(3), 807-826.
- 755 Fekiacova, Z., Pichat, S., Cornu, S., Balesdent, J., 2013. Inferences from the vertical distribution  
756 of Fe isotopic compositions on pedogenetic processes in soils. *Geoderma* 209, 110-118.
- 757 Fernandez, A., Borrok, D.M., 2009. Fractionation of Cu, Fe, and Zn isotopes during the  
758 oxidative weathering of sulfide-rich rocks. *Chemi. Geol.* 264 (1-4), 1-12.
- 759 Frierdich, A.J., Beard, B.L., Scherer, M.M., Johnson, C.M., 2014. Determination of the  
760 Fe(II)(aq)-magnetite equilibrium iron isotope fractionation factor using the three-isotope

- 761 method and a multi-direction approach to equilibrium. *Earth Planet. Sci. Lett.* 391, 77-  
762 86.
- 763 Frohne, T., Rinklebe, J., Diaz-Bone, R.A., Du Laing, G., 2011. Controlled variation of redox  
764 conditions in a floodplain soil: Impact on metal mobilization and biomethylation of  
765 arsenic and antimony. *Geoderma* 160(3-4), 414-424.
- 766 Fujii, T., Moynier, F., Abe, M., Nemoto, K., Albarede, F., 2013. Copper isotope fractionation  
767 between aqueous compounds relevant to low temperature geochemistry and biology.  
768 *Geochim. Cosmochim. Acta* 110, 29-44.
- 769 Fulda, B., Voegelin, A., Ehlert, K., Kretzschmar, R., 2013. Redox transformation, solid phase  
770 speciation and solution dynamics of copper during soil reduction and reoxidation as  
771 affected by sulfate availability. *Geochim. Cosmochim. Acta* 123, 385-402.
- 772 Garnier, J., J-M., G., C-L., V., Akerman, A., Chmeleff, J., Ruize, R.I., Poitrasson, F., 2017. Iron  
773 isotope fingerprints of redox and biogeochemical cycling in the soil-water-rice plant  
774 system of a paddy field. *Sci. Total Environ.* 574, 1622-1632.
- 775 Griffitt, R.J., Weil, R., Hyndman, K.A., Denslow, N.D., Powers, K., Taylor, D., Barber, D.S.,  
776 2007. Exposure to copper nanoparticles causes gill injury and acute lethality in zebrafish  
777 (*Danio rerio*). *Environ. Sci. Technol.* 41(23), 8178-8186.
- 778 Guelke, M., von Blanckenburg, F., Schoenberg, R., Staubwasser, M., Stuetzel, H., 2010.  
779 Determining the stable Fe isotope signature of plant-available iron in soils. *Chem. Geol.*  
780 277(3-4), 269-280.
- 781 Guilbaud, R., Butler, I.B., Ellam, R.M., Rickard, D., Oldroyd, A., 2011. Experimental  
782 determination of the equilibrium Fe isotope fractionation between Fe-aq(2+) and FeSm  
783 (mackinawite) at 25 and 2 degrees C. *Geochim. Cosmochim. Acta* 75(10), 2721-2734.
- 784 Hasselov, M., von der Kammer, F., 2008. Iron oxides as geochemical nanovectors for metal  
785 transport in soil-river systems. *Elements* 4(6), 401-406.
- 786 Hindersmann, I., Hippler, J., Hirner, A.V., Mansfeldt, T., 2014. Mercury volatilization from a  
787 floodplain soil during a simulated flooding event. *J. Soils Sed.* 14(9), 1549-1558.
- 788 Hindersmann, I., Mansfeldt, T., 2014. Trace element solubility in a multimetal-contaminated soil  
789 as affected by redox conditions. *Water Air Soil Poll.* 225(10).

- 790 Hofacker, A.F., Voegelin, A., Kaegi, R., Weber, F.A., Kretzschmar, R., 2013. Temperature-  
791 dependent formation of metallic copper and metal sulfide nanoparticles during flooding  
792 of a contaminated soil. *Geochim. Cosmochim. Acta* 103, 316-332.
- 793 Icopini, G.A., Anbar, A.D., Ruebush, S.S., Tien, M., Brantley, S.L., 2004. Iron isotope  
794 fractionation during microbial reduction of iron: The importance of adsorption. *Geology*  
795 32(3), 205-208.
- 796 Ilina, S.M., Poitrasson, F., Lapitskiy, S.A., Alekhin, Y.V., Viers, J., Pokrovsky, O.S., 2013a.  
797 Extreme iron isotope fractionation between colloids and particles of boreal and temperate  
798 organic-rich waters. *Geochim. Cosmochim. Acta* 101, 96-111.
- 799 Ilina, S.M., Viers, J., Lapitskiy, S.A., Mialle, S., Mavromatis, V., Chmeleff, J., Brunet, P.,  
800 Alekhin, Y.V., Isnard, H., Pokrovsky, O.S., 2013b. Stable (Cu, Mg) and radiogenic (Sr,  
801 Nd) isotope fractionation in colloids of boreal organic-rich waters. *Chem. Geol.* 342, 63-  
802 75.
- 803 IUSS Working Group WRB. 2014. World Reference Base for Soil Resources 2014. International  
804 soil classification system for naming soils and creating legends for soil maps. World Soil  
805 Resources Reports No. 106. FAO; Rome.
- 806 Johnson, C.M., Beard, B.L., Klein, C., Beukes, N.J., Roden, E.E., 2008. Iron isotopes constrain  
807 biologic and abiologic processes in banded iron formation genesis. *Geochim.*  
808 *Cosmochim. Acta* 72(1), 151-169.
- 809 Johnson, C.M., Roden, E.E., Welch, S.A., Beard, B.L., 2005. Experimental constraints on Fe  
810 isotope fractionation during magnetite and Fe carbonate formation coupled to  
811 dissimilatory hydrous ferric oxide reduction. *Geochim. Cosmochim. Acta* 69(4), 963-993.
- 812 Ju-Nam, Y., Lead, J.R., 2008. Manufactured nanoparticles: An overview of their chemistry,  
813 interactions and potential environmental implications. *Sci. Total Environ.* 400(1-3), 396-  
814 414.
- 815 Kayser, A., Presler, J., Meuli, R.G., Kägi, J., 2006. Bodenbelastungsgebiet Dornach -  
816 Synthesebericht, Amt für Umwelt des Kantons Solothurn, Solothurn.
- 817 Kiczka, M., Wiederhold, J.G., Frommer, J., Voegelin, A., Kraemer, S.M., Bourdon, B.,  
818 Kretzschmar, R., 2011. Iron speciation and isotope fractionation during silicate  
819 weathering and soil formation in an alpine glacier forefield chronosequence. *Geochim.*  
820 *Cosmochim. Acta* 75(19), 5559-5573.

- 821 Kimball, B.E., Mathur, R., Dohnalkova, A.C., Wall, A.J., Runkel, R.L., Brantley, S.L., 2009.  
822 Copper isotope fractionation in acid mine drainage. *Geochim. Cosmochim. Acta* 73(5),  
823 1247-1263.
- 824 Kusonwiriawong, C., Bigalke, M., Abgottspon, F., Lazarov, M., Wilcke, W., 2016. Response of  
825 Cu partitioning to flooding: A  $\delta^{65}\text{Cu}$  approach in a carbonatic alluvial soil. *Chem. Geol.*  
826 420, 69-76.
- 827 Lazarov, M., Horn, I., 2015. Matrix and energy effects during in-situ determination of Cu isotope  
828 ratios by ultraviolet-femtosecond laser ablation multicollector inductively coupled plasma  
829 mass spectrometry. *Spectrochim. Acta Part B* 111, 64–73.
- 830 Li, D.D., Liu, S.A., Li, S.G., 2015. Copper isotope fractionation during adsorption onto kaolinite:  
831 Experimental approach and applications. *Chem. Geol.* 396, 74-82.
- 832 Liu, S.A., Li, D.D., Li, S.G., Teng, F.Z., Ke, S., He, Y.S., Lu, Y.H., 2014a. High-precision  
833 copper and iron isotope analysis of igneous rock standards by MC-ICP-MS. *J. Anal. At.*  
834 *Spectrom.* 29(1), 122-133.
- 835 Liu, S.A., Teng, F.Z., Li, S.G., Wei, G.J., Ma, J.L., Li, D.D., 2014b. Copper and iron isotope  
836 fractionation during weathering and pedogenesis: Insights from saprolite profiles.  
837 *Geochim. Cosmochim. Acta* 146, 59-75.
- 838 Liu, K., Wu, L.L., Couture, R.M., Li, W.Q., Van Cappellen, P., 2015. Iron isotope fractionation  
839 in sediments of an oligotrophic freshwater lake. *Earth and Planetary Science Letters*, 423:  
840 164-172.
- 841 Lovley, D.R., 1991. Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiol. Rev.* 55(2), 259-287.
- 842 Malinovsky, D., Stenberg, A., Rodushkin, I., Andren, H., Ingri, J., Ohlander, B., Baxter, D.C.,  
843 2003. Performance of high resolution MC-ICP-MS for Fe isotope ratio measurements in  
844 sedimentary geological materials. *J. Anal. At. Spectrom.* 18(7), 687-695.
- 845 Mansfeldt, T., Overesch, M., 2013. Arsenic mobility and speciation in a gleysol with petrogleyic  
846 properties: A field and laboratory approach. *J. Environ. Qual.* 42(4), 1130-1141.
- 847 Mansfeldt, T., Schuth, S., Hausler, W., Wagner, F.E., Kaufhold, S., Overesch, M., 2012. Iron  
848 oxide mineralogy and stable iron isotope composition in a Gleysol with petrogleyic  
849 properties. *J. Soils Sed.* 12(1), 97-114.

- 850 Mathur, R., Ruiz, J., Titley, S., Liermann, L., Buss, H., Brantley, S., 2005. Cu isotopic  
851 fractionation in the supergene environment with and without bacteria. *Geochim.*  
852 *Cosmochim. Acta*, 69 (22), 5233-5246.
- 853 Mikutta, C., Wiederhold, J.G., Cirpka, O.A., Hofstetter, T.B., Bourdon, B., von Gunten, U.,  
854 2009. Iron isotope fractionation and atom exchange during sorption of ferrous iron to  
855 mineral surfaced. *Geochim. Cosmochim. Acta* 73, 1795-1812.
- 856 Moeller, K., Schoenberg, R., Pedersen, R.B., Weiss, D., Dong, S.F., 2012. Calibration of the new  
857 certified reference materials ERM-AE633 and ERM-AE647 for copper and IRMM-3702  
858 for zinc isotope amount ratio determinations. *Geostand. Geoanal. Res.* 36(2), 177-199.
- 859 Morgan, J.L.L., Wasylenki, L.E., Nuester, J., Anbar, A.D., 2010. Fe isotope fractionation during  
860 equilibration of Fe-organic complexes. *Environ. Sci. Technol.* 44(16), 6095-6101.
- 861 Mulholland, D.S., Poitrasson, F., Shirokova, L.S., Gonzalez, A.G., Pokrovsky, O.S., Boaventura,  
862 G.R., Vieira, L.C., 2015. Iron isotope fractionation during Fe(II) and Fe(III) adsorption  
863 on cyanobacteria. *Chem. Geol.* 400, 24-33.
- 864 Navarette, J.N., Borrok, D.M., Viveros, M., Ellzey, J.T., 2011. Copper isotope fractionation  
865 during surface adsorption and intracellular incorporation by bacteria. *Geochim.*  
866 *Cosmochim. Acta* 75, 784-799.
- 867 Oeser, M., Weyer, S., Horn, I., Schuth, S. 2014. High-precision Fe and Mg isotope ratios of  
868 silicate reference glasses determined in situ by femtosecond LA-MC-ICP-MS and by  
869 solution nebulisation MC-ICP-MS, *Geostand. Geoanalit. Res.* 37, 311-328.
- 870 Poitrasson, F., Viers, J., Martin, F., Braun, J.J., 2008. Limited iron isotope variations in recent  
871 lateritic soils from Nsimi, Cameroon: Implications for the global Fe geochemical cycle.  
872 *Chem. Geol.* 253(1-2), 54-63.
- 873 Pokrovsky, O.S., Vim, J., Emnova, E.E., Kompantseva, E.I., Freydier, R., 2008. Copper isotope  
874 fractionation during its interaction with soil and aquatic microorganisms and metal  
875 oxy(hydr) oxides: Possible structural control. *Geochim. Cosmochim. Acta* 72(7), 1742-  
876 1757.
- 877 Ponnamperna, F.N., 1972. The chemistry of submerged soils. *Advances in Agronomy* 24, 29-  
878 94.
- 879 Ratering, S., Schnell, S., 2000. Localization of iron-reducing activity in paddy soil by profile  
880 studies. *Biogeochemistry* 48(3), 341-365.



- 881 Reddy, T.R., Frierdich, A.J., Beard, B.L., Johnson, C.M., 2015. The effect of pH on stable iron  
882 isotope exchange and fractionation between aqueous Fe(II) and goethite. *Chemical*  
883 *Geology*, 397: 118-127.
- 884 Roh, Y., Zhang, C.L., Vali, H., Lauf, R.J., Zhou, J., Phelps, T.J., 2003. Biogeochemical and  
885 environmental factors in Fe biomineralization: Magnetite and siderite formation. *Clays*  
886 *Clay Miner.* 51(1), 83-95.
- 887 Ryan, B.M., Kirby, J.K., Degryse, F., Scheiderich, K., McLaughlin, M.J., 2014. Copper isotope  
888 fractionation during equilibration with natural and synthetic ligands. *Environ. Sci.*  
889 *Technol.* 48(15), 8620-8626.
- 890 Scherer, P., Lippert, H., Wolff, G., 1983. Composition of the major elements and trace elements  
891 of ten methanogenic bacteria determined by Inductively-Coupled Plasma Emission-  
892 Spectrometry. *Biol. Trace Elem. Res.* 5(3), 149-163.
- 893 Schuth, S., Hurraß, J., Münker, C., Mansfeldt, T., 2015. Redox-dependent fractionation of iron  
894 isotopes in suspensions of a groundwater-influenced soil. *Chem. Geol.* 392, 74-86.
- 895 Schuth, S., Mansfeldt, T., 2016. Iron isotope composition of aqueous phases of a lowland  
896 environment. *Environ. Chem.* 13(1), 89-101.
- 897 Tadanier, C.J., Schreiber, M.E., Roller, J.W., 2005. Arsenic mobilization through microbially  
898 mediated deflocculation of ferrihydrite. *Environ. Sci. Technol.* 39(9), 3061-3068.
- 899 Taylor, R.M., 1980. Formation and properties of Fe(II)Fe(III) hydroxy-carbonate and its possible  
900 significance in soil formation. *Clay Miner.* 15(4), 369-382.
- 901 Taylor, J.R. 1997. An introduction to error analysis: the study of uncertainties in physical  
902 measurements, 2nd ed. University Science Books, Sausalito, CA.
- 903 Teutsch, N., Schmid, M., Muller, B., Halliday, A.N., Burgmann, H., Wehrli, B., 2009. Large iron  
904 isotope fractionation at the oxic-anoxic boundary in Lake Nyos. *Earth Planet. Sci. Lett.*  
905 285(1-2), 52-60.
- 906 Teutsch, N., von Gunten, U., Porcelli, D., Cirpka, O.A., Halliday, A.N., 2005. Adsorption as a  
907 cause for iron isotope fractionation in reduced groundwater. *Geochim. Cosmochim. Acta*  
908 69(17), 4175-4185.
- 909 Thompson, A., Ruiz, J., Chadwick, O.A., Titus, M., Chorover, J., 2007. Rayleigh fractionation of  
910 iron isotopes during pedogenesis along a climate sequence of Hawaiian basalt. *Chem.*  
911 *Geol.* 238 (1-2), 72-83.

- 912 Vance, D., Archer, C., Bermin, J., Perkins, J., Statham, P.J., Lohan, M.C., Ellwood, M.J., Mills,  
913 R.A., 2008. The copper isotope geochemistry of rivers and the oceans. *Earth Planet. Sci.*  
914 *Lett.* 274(1-2), 204-213.
- 915 Weber, F.A., Voegelin, A., Kaegi, R., Kretzschmar, R., 2009a. Contaminant mobilization by  
916 metallic copper and metal sulphide colloids in flooded soil. *Nat. Geosci.* 2(4), 267-271.
- 917 Weber, F.A., Voegelin, A., Kretzschmar, R., 2009b. Multi-metal contaminant dynamics in  
918 temporarily flooded soil under sulfate limitation. *Geochim. Cosmochim. Acta* 73(19),  
919 5513-5527.
- 920 Weyer, S., Schwieters, J., 2003. High precision Fe isotope measurements with high mass  
921 resolution MC-ICPMS. *Int. J. Mass. Spectrom.* 226, 355–368.
- 922 Weyer, S., Anbar, A.D., Brey, G.P., Munker, C., Mezger, K., Woodland, A.B., 2005. Iron  
923 isotope fractionation during planetary differentiation. *Earth Planet. Sci. Lett.* 240(2), 251-  
924 264.
- 925 Wiederhold, J.G., Kraemer, S.M., Teutsch, N., Borer, P.M., Halliday, A.N., Kretzschmar, R.,  
926 2006. Iron isotope fractionation during proton-promoted, ligand-controlled, and reductive  
927 dissolution of goethite. *Environ. Sci. Technol.* 40(12), 3787-3793.
- 928 Wiederhold, J.G., Teutsch, N., Kraemer, S.M., Halliday, A.N., Kretzschmar, R., 2007a. Iron  
929 isotope fractionation during pedogenesis in redoximorphic soils. *Soil Sci. Soc. Am. J.*  
930 71(6), 1840-1850.
- 931 Wiederhold, J.G., Teutsch, N., Kraemer, S.M., Halliday, A.N., Kretzschmar, R., 2007b. Iron  
932 isotope fractionation in oxic soils by mineral weathering and podzolization. *Geochim.*  
933 *Cosmochim. Acta* 71(23), 5821-5833.
- 934 Wiesli, R.A., Beard, B.L., Johnson, C.M., 2004. Experimental determination of Fe isotope  
935 fractionation between aqueous Fe(II), siderite and "green rust" in abiotic systems. *Chem.*  
936 *Geol.* 211(3-4), 343-362.
- 937 Wu, L.L., Beard, B.L., Roden, E.E., Johnson, C.M., 2011. Stable iron isotope fractionation  
938 between aqueous Fe(II) and hydrous ferric oxide. *Environ. Sci. Technol.* 45(5), 1847-  
939 1852.
- 940 Zhu, X.K. et al., 2002. Mass fractionation processes of transition metal isotopes. *Earth Planet.*  
941 *Sci. Lett.* 200(1-2), 47-62.