

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

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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 μm) and colloidal fractions (0.02 - 10 μm). 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.

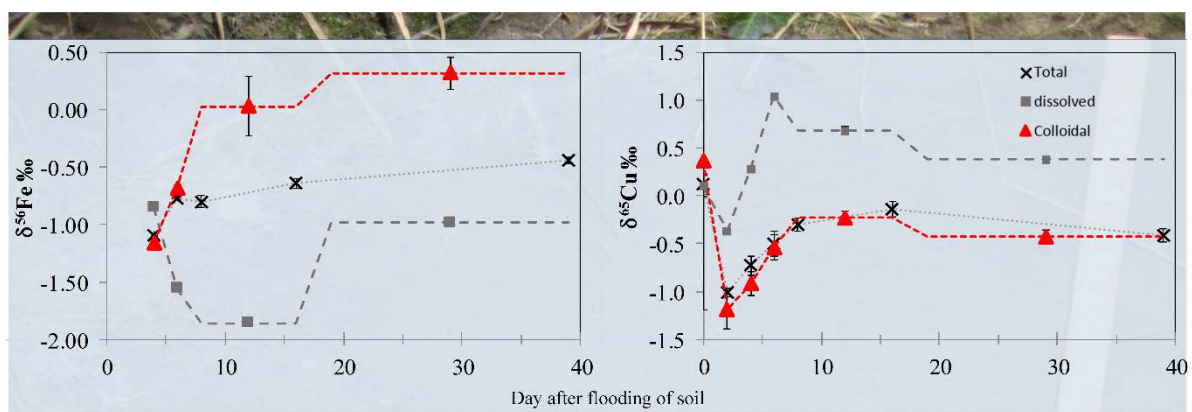
36 **Keywords: Copper, Iron, Soil, Redox Processes, Colloids, Stable Metal Isotopes**

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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.

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48 **Graphical Abstract**

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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 μm).
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 Fe^{3+} creates a reactive layer of Fe^{3+} at the oxide surface ($\text{Fe}^{3+}_{(\text{reac})}$) and is
109 associated with ^{56}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 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 μm 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}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 ± 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 ± 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 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 g kg^{-1} , a total Fe concentration of 12.6 g kg^{-1} and a dithionite-
177 extractable Fe concentration of 7.87 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 mg kg^{-1} (Kusonwiriawong 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‰ and
204 0.002‰) 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 HNO_3 (ROTH, Karlsruhe, Germany) and stored at -20°C prior to isotope analysis. The
218 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 H_2SO_4 (25%, p.a.),
220 0.5 mL of ammonium-acetate-acetic acid (400 g L^{-1} ammonium acetate, 50% acetic acid) and 0.2 mL
221 of phenanthroline solution (5 g L^{-1} 1,10-phenanthrolinechlorid) and made up to 10 mL with Millipore
222 water in the glovebox. Samples were measured after 15 minutes at the wavelength of 510 nm . A
223 detailed evolution of E_h , pH, SO_4^{2-} , S^{2-} , 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 mg of soil was digested
226 in HNO_3 , HF and H_2O_2 (3:2:1) in PFA beakers (Savillex, MN, USA) at least 24 h on a hot
227 plate at 120°C . The digested samples were evaporated until dryness at 70°C . Soil solution
228 samples were evaporated in PFA vials (Savillex, MN, USA). The dried residues were digested
229 using a mixture of concentrated HNO_3 and H_2O_2 (ratio 1:1) at 120°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 HNO_3 (3:1) at least 3 h at 120°C and finally dissolved in 7 mol L^{-1} HCl + 0.001%
232 H_2O_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 μ m) and dissolved
241 (<0.02 μ m) fractions because of too low mass (< 7 μ 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 (HNO₃ 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 Ω 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 ⁴⁰Ar¹⁴N⁺, ⁴⁰Ar¹⁶O⁺, and ⁴⁰Ar¹⁶OH⁺ on ⁵⁴Fe, ⁵⁶Fe, and ⁵⁷Fe (Weyer and
259 Schwieters, 2003). Chromium (⁵³Cr) was monitored to correct isobaric interferences of ⁵⁴Cr
260 on ⁵⁴Fe. 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⁻¹ 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⁻¹ Fe with 2%
266 HNO₃. 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, R²= 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[®], 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±0.03‰ (mean ±2SD, n=5) which is
278 identical to the pure Merck Fe value (0.19±0.02‰, n=3). The USGS basalt BCR-2 yielded a
279 mean $\delta^{56}\text{Fe}$ value of 0.07±0.02‰ (2SD, n=4) which agrees well with previously published
280 values ranging from 0.05±0.02‰ to 0.11±0.03‰ (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±0.02‰ (mean ± 2SD, n=6) and again in good agreement with previously
283 published values varying from -0.71±0.18‰ to -0.73±0.10‰ (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 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[®]-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[®]-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 δ values of the composite samples,

313 we averaged the values of the colloidal concentrations and the δ values based on mass balance
 314 approaches, as shown in Equation 2 with δ_{ss} and C_{ss} being the delta value and the
 315 concentration of the respective subsample. In Equation 1-5 δ_{tot} , δ_{diss} and δ_{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 δ 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 δ 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 μm), dissolved (<0.02 μm) and (calculated)
 331 colloidal (0.02-10 μm) 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	δ value (‰)	2SD	n ^a	Conc. ($\mu\text{mol L}^{-1}$)	SD	δ value (‰)	2SD	n ^a	Conc. ^h ($\mu\text{mol L}^{-1}$)	SD ^e	δ value ^f (‰)	2SD ^g
Fe	Soil	226000 ^b	22920 ^b	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 ^c	19.8	-1.86 ^b	0.03	1	197 ^c	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 ^d	24.3	-0.98 ^c	0.01	1	203 ^d	25.9	0.32	0.14
Cu	Soil	1920 ^b	70 ^b	-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 ^c	0.03	0.68 ^c	0.04	1	10.1	1.37	-0.23	0.07
39	2.64	0.82	-0.41	0.07	3	0.07 ^d	0.02	0.38 ^d	0.03	1	2.59	0.82	-0.43	0.07	

332 ^aNumber of replicate preparations and analysis, ^bConcentrations in the solid soil are in $\mu\text{mol kg}^{-1}$, ^cBulked sample combined from days 8 to 16,

333 ^dBulked sample combined from days 19 to 39, ^eCalculated by error propagation of standard deviation in total and dissolved concentration,

334 ^fCalculated by the difference between the $\delta^{65}\text{Cu}$ values of total and dissolved Cu (Eq. 1), ^gCalculated by error propagation according to Eq. S1-S4,

335 ^hCalculated by subtracting the dissolved from the total concentrations.

336 3. Results

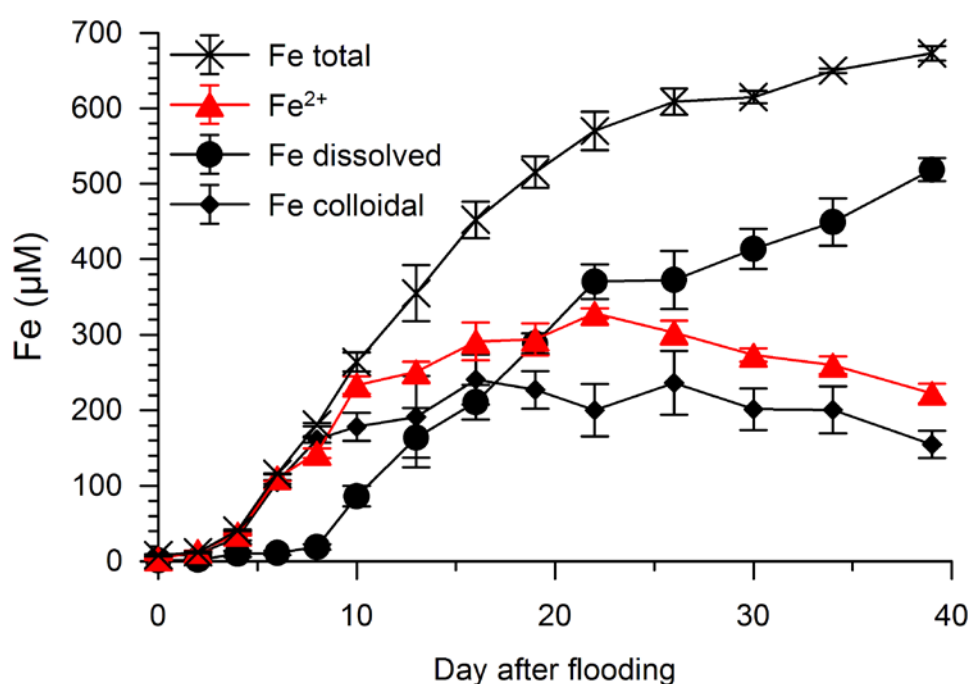
337 Iron was first mainly released in colloidal form, which changed after 19 days to
338 predominantly dissolved Fe (Figure 1 and Table S3, Abgottspon et al., 2015). On day 39, only
339 22% of the total Fe was still in colloidal form. The sum of total and dissolved Fe
340 concentrations increased continuously 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 Fe²⁺, but the concentration of Fe²⁺ started to deviate
343 substantially from that of total Fe from day 13 on. After day 13, the Fe²⁺ 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 Fe²⁺ indicating that Fe³⁺ was
346 also present in the dissolved phase. On day 29, only 33% of the total Fe in solution was still
347 Fe²⁺.

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 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 $-0.08 \pm 0.04\text{‰}$ ($n=3$).

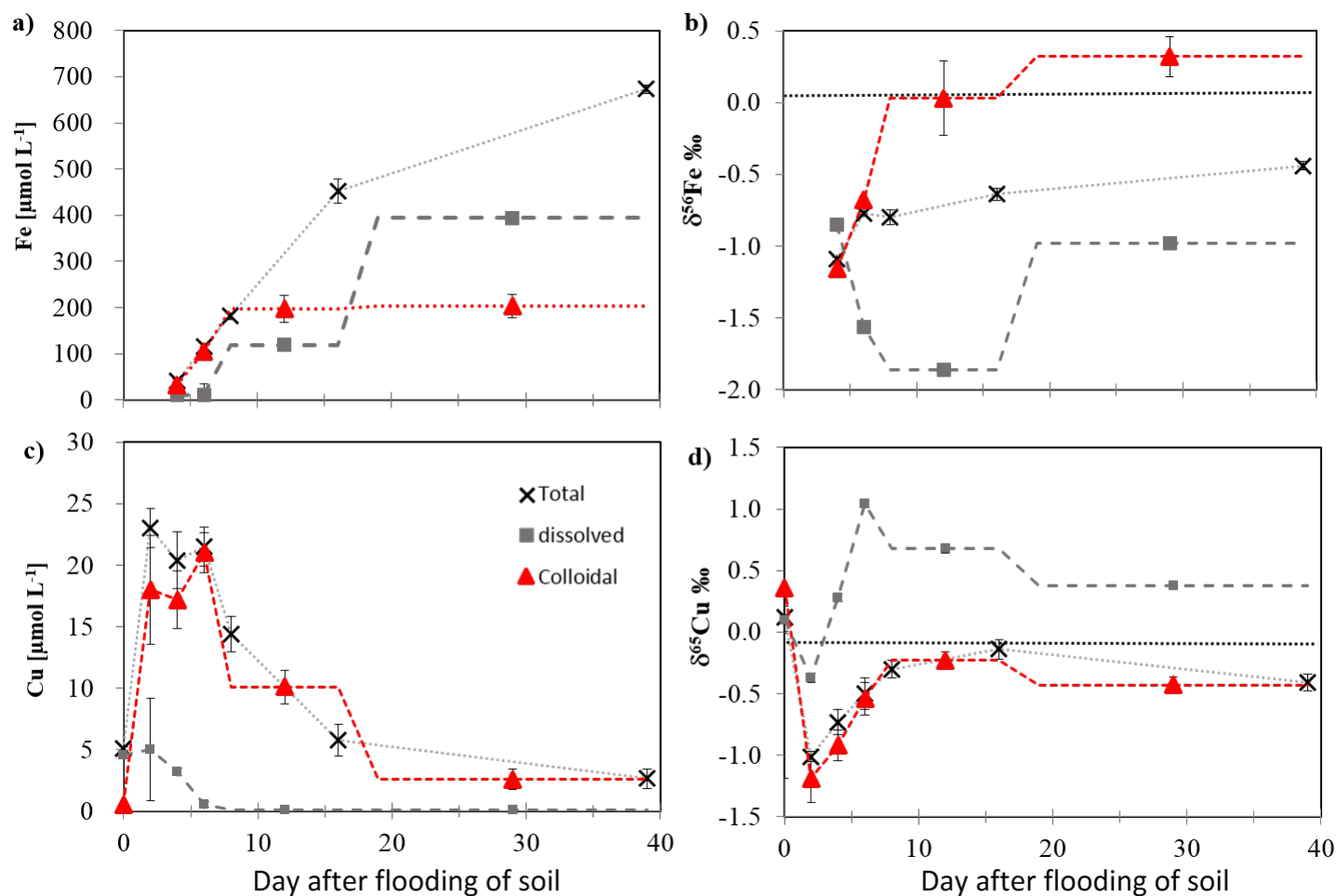
373



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 Fe^{2+} concentrations
 377 were specifically measured for this study in unfiltered samples and therefore represent total Fe^{2+} in
 378 solution including dissolved and colloidal fractions.

379



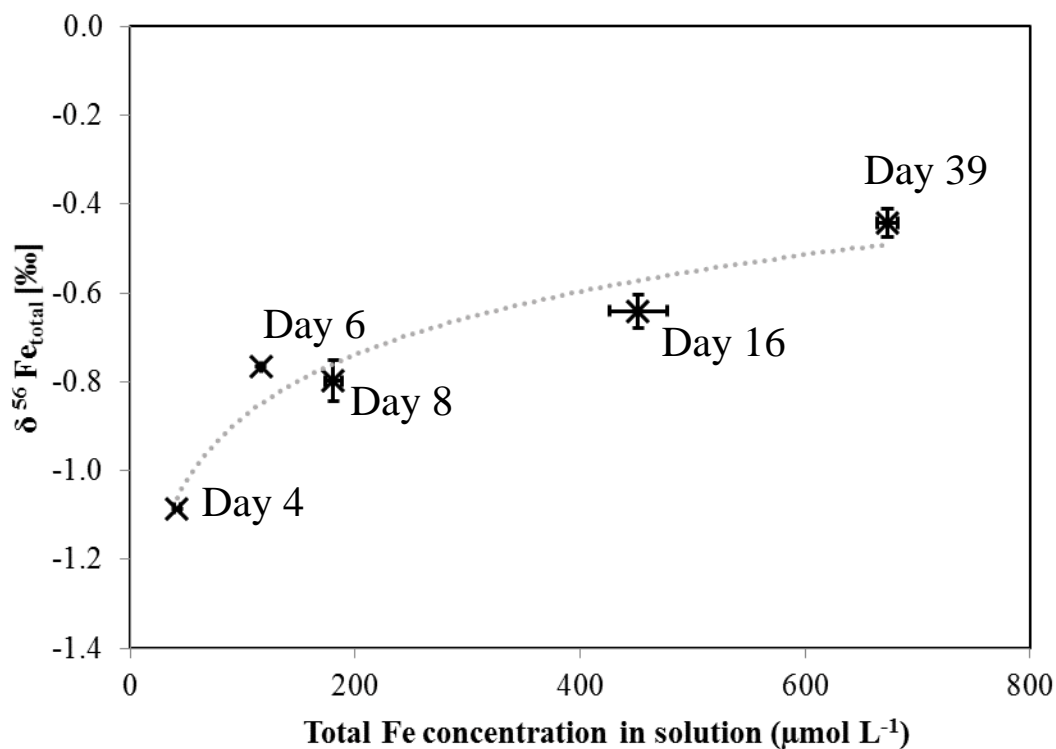
380

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 ± 0.01 ‰) and $\delta^{65}\text{Cu}$ ($-$
 384 0.08 ± 0.04 ‰) 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).



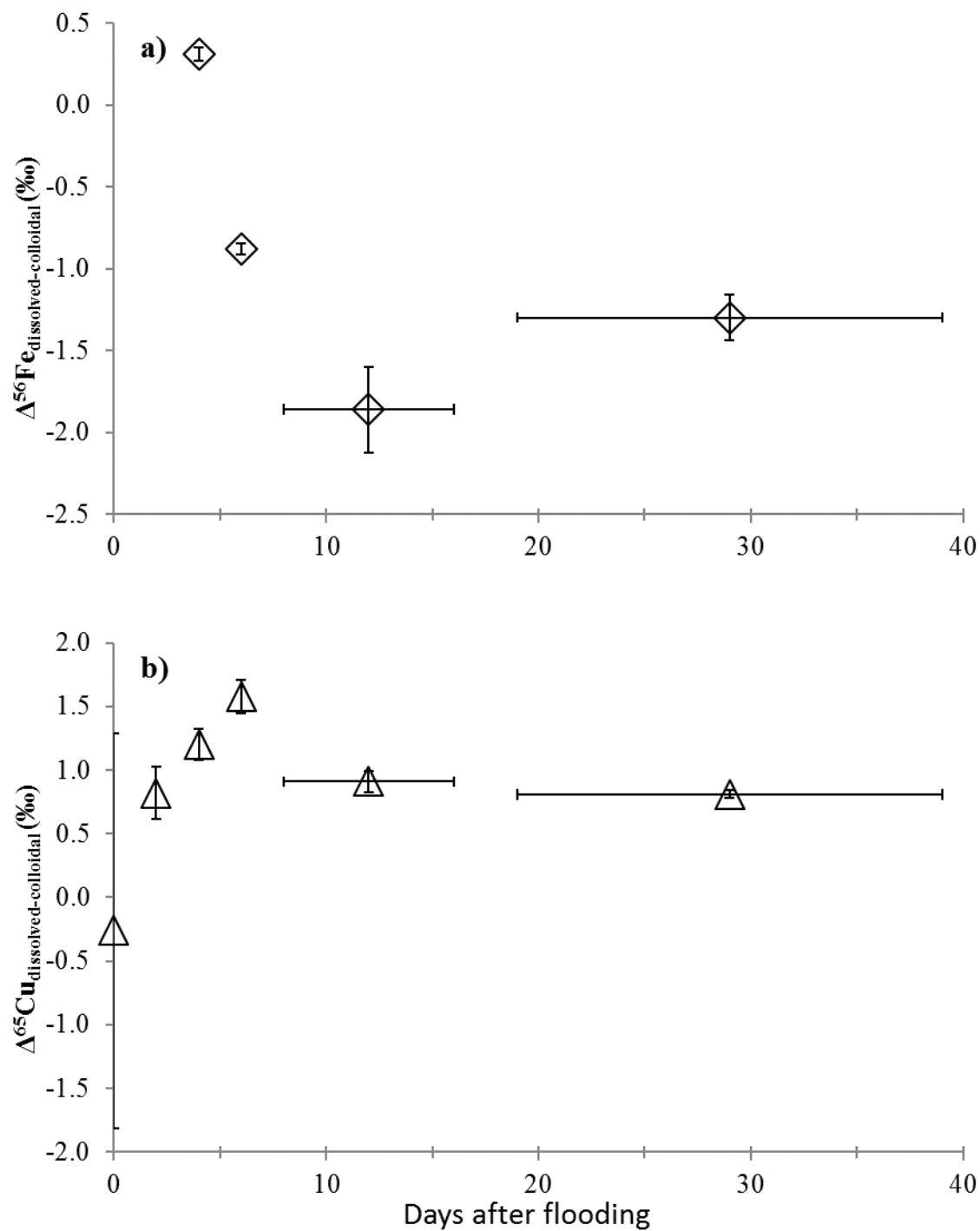
398

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 Fe^{2+} , while the contribution of Fe^{2+} to total
404 Fe in solution decreased after day 10 (Fig. 1), which is similar to the increase in Fe^{3+}
405 concentrations in solution reported by Schuth et al. (2015). After day 19, total dissolved Fe
406 showed higher concentrations than Fe^{2+} , indicating the presence of Fe^{3+} even in the dissolved
407 phase. Because the solubility of Fe^{3+} is very low, this finding may only be explained by small
408 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 Fe^{3+} by organic substances, forming soluble
410 complexes. Another possible explanation might be an analytical complication in the
411 determination of the 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 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^{2+} 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 $\text{Fe}^{2+}_{\text{aq}}$
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 ± 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^{2+} 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 Fe^{2+} produced by DIR and 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 mmol L^{-1} on day 4, Abgottspon et al.,
456 2015) might cause a precipitation of FeCO_3 directly from dissolved Fe^{2+} and 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 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 Fe^{2+} to Fe^{3+} in the soil
472 solution and thus changing proportions of Fe^{2+} and 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 Fe^{2+} indicate that colloids change from a Fe^{2+} dominated mineral to
475 a mineral containing also 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; NH_4NO_3 -
496 extractable, NaOAc-extractable, NH_4Ox -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 Fe^{2+} analysis. This separation is hardly possible
532 under oxic field conditions as reduced 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}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 Cu^+ , which disproportioned into Cu^{2+} , released into solution, and zero-valent
561 Cu (Cu^0) colloids, in this phase of the incubation. In solution, 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 Cu_xS (Weber et al., 2009b). If this process also occurred
564 in our experiment the Cu_xS 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 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 mmol L^{-1}
582 C_{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 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 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 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‰ 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}Cu and their precipitation results in the enrichment of ^{65}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 (Kusonwiriawong 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; NH_4NO_3 -
613 extractable, NaOAc-extractable, NH_4Ox -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 Kusonwiriawong 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 Cu_xS 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‰) 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

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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

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