Doctoral Thesis

Changes in copper and cadmium solubility and speciation induced by soil redox dynamics
Competitive metal sulfide formation and interactions with natural organic matter

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Changes in copper and cadmium solubility and speciation induced by soil redox dynamics - Competitive metal sulfide formation and interactions with natural organic matter

A dissertation submitted to
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DOCTOR OF SCIENCE

presented by
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Summary

The accumulation of trace metals from anthropogenic emissions in periodically flooded soils, like riparian floodplain or paddy soils, raised the concern of their potential release to adjacent surface and groundwater resources and their transfer into the human food chain. Reliable estimations of the respective risks require a detailed mechanistic understanding of the processes controlling trace metal solubility in these environmental systems. Periodically flooded soils are characterized by strong variations in soil redox conditions, which influence trace metal sorption-desorption and precipitation-dissolution reactions. The microbial reduction of sulfate to sulfide and subsequent precipitation of chalcophile trace metals as sparingly soluble sulfides is considered to be a key process for the sequestration of potential toxic trace metals during periods of high water levels, and may further influence their remobilization potential upon soil reoxidation. However, the sulfur content of freshwater wetland soils is in general low, which implies that if these soils are highly contaminated the amount of trace metals may exceed the present pool of reducible sulfate. In previous studies it was suggested that under such conditions, the extent to which individual metals are sequestered and potentially immobilized in metal sulfides depends on the thermodynamic stability of their respective sulfide minerals (e.g., CuS > CdS) as well as on the kinetics of metal resupply and sulfide precipitation. The aim of this thesis was to investigate redox-induced changes in copper (Cu) and cadmium (Cd) solubility and solid-phase speciation in periodically flooded soils during soil reduction and subsequent reoxidation with special focus on competitive metal sulfide formation and interactions with natural organic matter.

Laboratory soil incubation experiments with a Cu and Cd spiked paddy soil at various metal-to-sulfate ratios revealed that Cu and Cd solubility and solid-phase speciation during soil reduction and reoxidation is influenced by several factors and processes. Cu as a redox active metal was rapidly reduced from Cu(II) to Cu(I) and Cu(0). X-ray absorption spectroscopy results suggested
that in the absence of sufficient amounts of biogenic sulfide, i.e. prior to sulfate reduction or at high metal-to-sulfate ratios, Cu(I) was stabilized by binding to reduced organic sulfur groups \( S_{\text{org}} \) of natural organic matter. Additional model experiments, investigating Cu redox transformation and complexation by reduced and oxidized soil humic acid (HA), revealed that Cu(I) binding by HA likely involves the contribution of nitrogen and sulfur ligands. It was further shown that HA can act as an electron donor and acceptor for Cu redox transformation, which is a possible pathway for the formation of Cu(I) and Cu(0) in soils. In the presence of moderate to high reducible sulfate amounts, formation of amorphous Cu-sulfide phases was observed during major sulfate reduction. Although, Cu was found to be the dominant sulfide forming metal under all sulfate scenarios, X-ray absorption spectroscopy results suggested the formation of nanometer-sized and/or poorly crystalline Cd-sulfide even at very high metal-to-sulfate ratios. The stronger adsorption of Cu in soils compared to Cd and to some extent also the formation of Cu(I)-S\(_{\text{org}}\) and Cu(0) may have attenuated the competitive effect of Cu on Cd during sulfide precipitation. Nevertheless, the decrease in Cd solubility and extractability during soil reduction was shown to be closely related to the initial metal-to-sulfate ratios. Under strongly sulfate limited conditions, Cd remained in a labile form over several weeks of soil flooding. Upon reaeration, Cu(0) and Cd-sulfide were rapidly oxidized while oxidative dissolution of Cu-sulfide appeared to be rather slow. Therefore, the remobilization potential of Cu and Cd upon soil reoxidation differs and is at least for Cu influenced by the solid phase speciation of the previous reduction period.

The results presented in this thesis demonstrate that the dynamics of trace metals in multi-metal contaminated soils under fluctuating redox conditions is a complex interplay of their specific biogeochemical behavior, i.e. redox activity, sorption affinity, and metal sulfide stability, as well as depends on soil chemical factors like the amount of reducible sulfate and factors that influence the reductive processes in the soil, i.e. duration of flooding period or temperature. The observed transformation processes and dynamics of Cu and Cd may provide valuable information for the prediction of trace metal mobilization in multi-metal contaminated floodplain soils as well as for the risk assessment of the soil-plant transfer of trace metals in agricultural used wetlands, like paddy soils.
Zusammenfassung

Zusammenfassung

unterschiedlich und kann, zumindest für Cu, durch die während der Reduktion gebildeten Festphasen bestimmt werden.

Die in dieser Dissertation präsentierten Resultate zeigen, dass die Schwermetalldynamik in kontaminierten Böden unter wechselnden Redoxbedingungen ein komplexes Zusammenspiel verschiedener Faktoren ist: Dazu gehören das biogeochemische Verhalten einzelner Schwermetalle, d.h. deren Redoxaktivität, Bindungsvermögen im Boden und die Stabilität ihrer Metallsulfide, sowie bodenchemische Faktoren, wie die Verfügbarkeit von reduzierbarem Sulfat und Faktoren, die die Reduktionsprozesse im Boden beeinflussen, wie die Überflutungsdauer oder das Temperaturregime. Die in dieser Arbeit aufgezeigten Umwandlungsprozesse und die damit verbundene Dynamik von Cu und Cd können einen wichtigen Beitrag für die Vorhersage der Schwermetallmobilisierung in kontaminierten periodisch gefluteten Böden leisten, sowie Anwendung in der Gefahrenbewertung der Aufnahme von Schwermetallen in Pflanzen in landwirtschaftlich genutzten, redoxbeeinflussten Böden (z.B. Reisfelder), finden.
1. Introduction

1.1. Research motivation

During the last century, industrialization and the fast growing global population caused an increasing demand for energy and natural sources as well as the need for intensified food production. This led to a worldwide increase of anthropogenic emissions of potential toxic trace metals and metalloids (e.g. As, Ag, Cd, Co, Cr, Cu, Hg, Ni, Pb, Sb, Zn) into the environment. The anthropogenic sources of trace metals range from mining and smelting, fossil fuel combustion, urban and industrial sewage discharge, and the extensive use of pesticides and fertilizers in agriculture. Trace metals are released and transported in the environment in gaseous, aqueous, particulate, or solid form and can contaminate soils, surface water bodies and subsurface aquifers (Alloway, 1995; Adriano, 2001). Some trace metals (e.g., Cu, Co, Ni, Zn) are essential micronutrients for living organisms, but at higher concentrations all trace metals are toxic to plants, microorganisms, animals and humans (Adriano, 2001). Depending on the solubility, mobility and bioavailability of trace metals in contaminated environmental systems, they can pose a threat for soil fertility, ecosystem functioning and human health.

Wetland soils, i.e. soils that are intermittently or permanently submerged, play an important role in the cycling of trace metals in the environment, as they can act as sinks and sources for trace metals (Gambrell, 1994; Kirk, 2004; Du Laing et al., 2009). Of special interest in this context are riparian floodplain soils, which can receive high loads of dissolved and particle-bound trace metals that are transported with the river water and are deposited in the floodplain during flooding periods (Du Laing et al., 2009). As a result, high levels of trace metals have been reported for many floodplain soils along major European rivers and their tributaries (Table 1.1). Although, floodplains are important sinks for trace metals, the may also act as sources when the concentrations of trace metals in the river water are decreasing or when hydraulic and
geochemical conditions significantly change (Schulz-Zunkel and Krueger, 2009) due to restoration of former wetlands (RAMSAR, 2010) or climate change (Houghton et al., 2001; Booij, 2005). The mobility and bioavailability of trace metals therefore not only affect floodplain ecosystem health itself but also the quality of adjacent surface and groundwater resources (Olivie-Lauquet et al., 2001). The use of polluted floodplains as grassland for production of hay or as pasture for cattle grazing also raises the concern of a transfer of trace metals into the human food chain (Overesch et al., 2007). This is particularly relevant for wetland soils used for intensive agriculture like paddy soils. High contamination with trace metals has been reported for paddy soils related to the use of contaminated irrigation water, e.g., in mining affected areas, or due to the application of contaminated fertilizers and pesticides (Gimeno-Garcia et al., 1996; Kashem and Singh, 1999; Lee, 2006; Williams et al., 2009). The uptake of trace elements like As and Cd into the rice plant and translocation into the edible parts poses a serious human health threat and has therefore become an important food safety issue in many Asia countries, where rice is a staple crop (Yang et al., 2006; Rahman et al., 2009; Uraguchi et al., 2009; Dittmar et al., 2010). In addition, the contamination of arable soils can lead to reduced yields, as most trace metals are toxic to plants at high concentrations (Bingham et al., 1980; Cao and Hu, 2000; Panaullah et al., 2009).

Riparian floodplain soils and paddy soils are characterized by periodic flooding and variations in groundwater level. The associated changes in soil redox state have important consequences for trace metal dynamics in these soils (Gambrell, 1994; Du Laing et al., 2009; Borch et al., 2010). Over the past decades, extensive research has been conducted for the assessment of trace metal behavior in fully oxidized soils (Sparks, 2001) and permanently reducing subsurface environments, i.e. sediments, where metal sulfide formation was found as the major factor controlling trace metal speciation (Huerta-Diaz et al., 1998; Morse and Luther, 1999; Billon et al., 2001). However, our understanding of the biogeochemical processes controlling trace metal chemistry in ecosystems subject to fluctuating redox conditions at different temporal and spatial scales is still limited (Borch et al., 2010). The following section gives a short introduction in the biogeochemistry of periodically flooded wetlands soils and key processes affecting trace metal dynamics.
1.2. Trace metal cycling in wetland soils

Periodic flooding and related reduction and oxidation processes can strongly affect trace metal speciation and solubility in wetland soils. Varying soil redox conditions influence trace metal sorption-desorption and precipitation-dissolution reactions and may also induce changes in the redox-state of redox-sensitive trace elements (Du Laing et al., 2009; Borch et al., 2010).

During soil flooding, microbial respiration of organic carbon leads to rapid depletion of \( \text{O}_2 \) and the sequential reduction of alternative electron acceptors (nitrate, Mn(IV,III) and Fe(III) (oxyhydr)oxides, sulfate) (Ponnamperuma, 1972; Kirk, 2004). In weakly acid wetland soils, these reduction processes lead to an increase of soil pH to near-neutral conditions, which significantly enhances trace metal sorption to organic matter (OM), clay and oxide minerals (Christl et al., 2001; Fischer et al., 2007; Gu et al., 2010). In contrast, the reductive dissolution of Mn(IV,III) and Fe(III) (oxyhydr)oxides can cause the release of adsorbed or co-precipitated trace metals.

### Table 1.1. Examples of trace metal contamination in riparian floodplain soils and paddy soils in various countries in comparison to average world-soil background concentrations.

<table>
<thead>
<tr>
<th>Wetland soils</th>
<th>Cd (mg kg(^{-1}))</th>
<th>Cu (mg kg(^{-1}))</th>
<th>Ni (mg kg(^{-1}))</th>
<th>Pb (mg kg(^{-1}))</th>
<th>Zn (mg kg(^{-1}))</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riparian floodplains</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elbe (Germany)</td>
<td>0.1-30</td>
<td>8.8-540</td>
<td>7.0-140</td>
<td>11.370</td>
<td>34-2500</td>
<td>(a)</td>
</tr>
<tr>
<td>Mulde (Germany)</td>
<td>30</td>
<td>279</td>
<td>106</td>
<td>408</td>
<td>1370</td>
<td>(b)</td>
</tr>
<tr>
<td>Dommel (Netherlands)</td>
<td>6.7-70</td>
<td>26-108</td>
<td>16-38</td>
<td>105-209</td>
<td>327-1470</td>
<td>(c)</td>
</tr>
<tr>
<td>Rhine, Meuse (Netherlands)</td>
<td>ns</td>
<td>30-130</td>
<td>ns</td>
<td>70-490</td>
<td>170-1450</td>
<td>(d)</td>
</tr>
<tr>
<td>Hamps, Manifold (UK)</td>
<td>0.3-22</td>
<td>11.5-318</td>
<td>17-81</td>
<td>16-1108</td>
<td>92-6391</td>
<td>(e)</td>
</tr>
<tr>
<td>Odra (Poland)</td>
<td>2.0-10</td>
<td>ns</td>
<td>ns</td>
<td>30-1500</td>
<td>240-2500</td>
<td>(f)</td>
</tr>
<tr>
<td>Seine (France)</td>
<td>130</td>
<td>347</td>
<td>150</td>
<td>558</td>
<td>1620</td>
<td>(g)</td>
</tr>
<tr>
<td>Paddy soils</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tak Province (Thailand)</td>
<td>0.5-284</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>100-8036</td>
<td>(h)</td>
</tr>
<tr>
<td>Guixi smelting area (China)</td>
<td>0.2-13</td>
<td>87-548</td>
<td>2.6-18</td>
<td>11-40</td>
<td>45-430</td>
<td>(i)</td>
</tr>
<tr>
<td>Duckum mining area (Korea)</td>
<td>7.0-15</td>
<td>9.0-71</td>
<td>11-24</td>
<td>24-1200</td>
<td>83-861</td>
<td>(j)</td>
</tr>
<tr>
<td>Sambo mining area (Korea)</td>
<td>0.6-7.3</td>
<td>1-103</td>
<td>ns</td>
<td>17-408</td>
<td>74-3100</td>
<td>(k)</td>
</tr>
<tr>
<td>World-soil average</td>
<td>0.41</td>
<td>38.9</td>
<td>29</td>
<td>27</td>
<td>70</td>
<td>(l)</td>
</tr>
</tbody>
</table>

ns = not specified. (a) Krueger and Groengroeft (2003), (b) Weber et al. (2009a), (c) Poot et al. (2007), (d) Middelkoop (2000), (e) Bradley and Cox (1986), (f) Ciszewski et al. (2008), (g) Meybeck et al. (2007), (h) Simmons et al. (2005), (i) Hu et al. (2006), (j) Lee (2006), (k) Jung and Thornton (1997), (l) (Kabata-Pendias, 2011).
Chapter 1

metals, which is enhanced by the increase in dissolved Mn$^{2+}$ and Fe$^{2+}$ that compete for sorption sites (Zachara et al., 2001; Weber et al., 2009a; Hofacker et al., 2013). The release of dissolved organic matter (DOM) as a result of increasing soil pH and microbial decomposition of soil organic matter (SOM) can mobilize organically-complexed trace metals into the soil solution (Grybos et al., 2007; Grybos et al., 2009).

The mobilization of trace metals in solution can be counteracted by precipitation, coprecipitation and adsorption processes. Microbial respiration results in the accumulation of dissolved carbonate. Together with the increasing concentrations of dissolved Fe$^{2+}$ and Mn$^{2+}$, and related release of Ca$^{2+}$ and Mg$^{2+}$, the soil solution can become oversaturated with respect to single or mixed carbonate phases over extended periods of submergence (Jensen et al., 2002; Kirk, 2004). Trace metals may be incorporated into or adsorbed to these carbonate phases (Rimstidt et al., 1998; Elzinga et al., 2006), form surface precipitates (Comans and Middelburg, 1987), or precipitate as pure trace metal carbonate phases (Khaokaew et al., 2011).

Furthermore, green rust (Fe(II)-Fe(III) layered double hydroxides) is considered to be an important mineral phase in soils exposed to fluctuating redox conditions (Feder et al., 2005), which may be capable of incorporating significant quantities of certain trace metals (Parmar et al., 2001). Under sulfate-reducing conditions, the formation of hydrogen sulfide may decrease trace metal solubility, due to the precipitation of chalcophile trace metals as sparingly soluble metal sulfides or due to their co-precipitation with Fe-sulfides in sulfur-rich sediments (Huerta-Diaz et al., 1998; Morse and Luther, 1999; Billon et al., 2001).

Redox sensitive trace elements like As, Cu, Cr, Hg and Ag may also be affected by direct redox transformations mediated by microorganisms (Lovley, 1993), chemical reaction with dissolved (Matocha et al., 2005), adsorbed (Genovese and Mellini, 2007) or solid state Fe(II) in mineral phases like green rust (Williams and Scherer, 2001; O’Loughlin et al., 2003), or by redox-active functional groups of natural organic matter (see also section 1.5) (Palmer et al., 2006; Gu et al., 2011; Maurer et al., 2012; Pham et al., 2012).

Upon soil reaeration, trace metals are redistributed due to the oxidative processes that occur in contact with oxygen. Studies on the oxidation of sulfide-rich sediments have shown a mobilization of trace metals due to the oxidative dissolution of metal-sulfides (Carroll et al.,
1.3 Copper and cadmium in soils

Contaminated soils are typically affected by multiple rather than a single trace metal. The fate of each metal in the soil is therefore not only influenced by its interaction with major soil components, but also by competitive interactions with other trace elements during adsorption and precipitation reactions. In this section a short comparative overview on the role and biogeochemistry of Cu and Cd in soils is presented.

Cu and Cd occur naturally in the environment in igneous and sedimentary rocks at average concentrations of 55 and 0.1 mg kg\(^{-1}\), respectively, and are released during natural rock weathering (Kabata-Pendias, 2011). Cu is found predominantly into sulfidic minerals, dominantly chalcopyrite (CuFeS\(_2\)), and is incorporated in secondary oxide and carbonate minerals during weathering. It is also often associated with other sulfide minerals like sphalerite (ZnS), pyrite (FeS\(_2\)) and galena (PbS). Cu can also occur as native metal. Common Cd minerals are, e.g., greenockite (CdS), otavite (CdCO\(_3\)) and monteponite (CdO). Cd is geochemically closely related to Zn and is therefore often associated with Zn, Pb-Zn and Pb-Cu-Zn ores (Adriano, 2001). Significant concentrations of Cd are also found in phosphate ores, especially in
sedimentary phosphate rocks that are widely used as phosphate fertilizers (McLaughlin and Singh, 1999).

Natural background concentrations for Cu and Cd in uncontaminated soils are in the range of 14–109 mg kg\(^{-1}\) and 0.2–1.1 mg kg\(^{-1}\), respectively (Kabata-Pendias, 2011). Elevated Cd concentrations in soils are most often related to anthropogenic pollution. Important anthropogenic sources for Cd in soils are Pb and Zn mining and smelting, where Cd is released as a by-product, and the application of Cd-contaminated phosphate fertilizers in agriculture (McLaughlin and Singh, 1999). Cd concentrations in riparian floodplain or paddy soils in mining affected areas can reach very high values (Table 1.1). For example, Cd concentrations of up to 284 mg kg\(^{-1}\) were reported for paddy fields downstream of a Zn mining area in western Thailand (Simmons et al., 2005). Important anthropogenic sources for Cu, besides mining and smelting, are sewage discharge, the wide use of Cu as a biocide and the release from industrial sources (Adriano, 2001). Elevated Cu concentrations in river floodplain soils have therefore been reported for highly industrialized areas, e.g., the industrial district of Bitterfeld-Wolfen (Germany) or the River Seine catchment (Kalbitz and Wennrich, 1998; Meybeck et al., 2007).

The biological role of Cu and Cd in soils is very different. Cu is an essential element for living organisms and is required for the viability of a variety of proteins and enzymes that carry out fundamental biological functions (Rubino and Franz, 2012). For example, Cu is involved in important biogeochemical cycles such as methane oxidation and denitrification (Averill, 1996; Semrau et al., 2010). Cu deficiency in agricultural soils causes reduced yield and quality of crop products, and has been reported for many areas of the world (Alloway, 1995). However, elevated copper concentrations are toxic to most bacteria and plants and can therefore adversely affect ecosystem functioning (Baath, 1989; Fernandes and Henriques, 1991). Cd on the other side, is a non-essential trace metal, which adversely affects biological processes of humans, animals and plants (Kabata-Pendias, 2011). Cd can accumulate in plants to concentrations that are not phytotoxic, but yet pose a serious human health risk (McLaughlin et al., 1999). The accumulation of Cd in the human food chain is of great concern with respect to rice production on Cd-contaminated paddy soils (Chaney et al., 1996) as daily ingestion of Cd-contaminated rice causes severe health problems such as itai-itai disease (Nordberg, 2004).
The bioavailability and toxicity of Cu and Cd in soils strongly depends on their speciation and solubility, which largely varies under oxic and anoxic conditions. Under oxic conditions Cu$^{2+}$ and Cd$^{2+}$ are both adsorbed to Fe, Mn and Al (oxyhydr)oxides, clay minerals and NOM (McBride, 1989). The adsorption of Cu and Cd to these soil compounds is strongly pH-dependent and increases and becomes more specific with increasing pH. In comparison to Cd, Cu has a much higher adsorption affinity for clay and oxide mineral surfaces (McBride, 1989; Fischer et al., 2007) and especially NOM (Christl et al., 2001; Maurer et al., 2012). At weakly acid to neutral conditions, Cd is therefore considered to be much more mobile and bioavailable than Cu. This implies that in multi-metal contaminated soils, Cd adsorption may be influenced by the presence of Cu. Competitive sorption experiments for Cu and Cd on soil clay minerals showed that increasing concentrations of strongly bound Cu reduced the total and specific adsorption of Cd (Atanassova, 1999).

Under anoxic conditions, the speciation of Cu and Cd in the soils changes significantly compared to oxic conditions. Cu and Cd are both chalcophile trace elements that exhibit a high affinity for sulfur. Under strongly reducing conditions, Cu and Cd can therefore both form sparingly soluble metal sulfides, which are considered to reduce their mobility and bioavailability (Di Toro et al., 1990; Berry et al., 1996; Morse and Luther, 1999). The thermodynamic stability of CuS, however, is significantly higher than that of CdS (Table 1.2). For multi-metal contaminated floodplain soils with low sulfate contents, it was therefore suggested that the concentration of Cu relative to reducible sulfate may control whether CdS forms or not, although also the kinetics of metal resupply and sulfide precipitation need as well to be considered (Weber et al., 2009a). The behavior of Cu and Cd under reducing conditions may also differ due to the fact that Cu is a redox active trace metal and can be reduced from Cu(II) to Cu(I) or even Cu(0). Reduced forms of Cu are expected to largely differ from Cu(II) with respect to their biogeochemical reactivity. For example, Cu(I) is expected to have a higher affinity for sulfur-containing ligands of natural organic matter than Cu(II) due to its softer character (Smith et al., 2002), as discussed in more detail in section 1.5. Metallic Cu(0) formation has been reported for a contaminated floodplain soil as an intermediate phase during initial stage of soil reduction, which temporarily enhanced
the mobility of Cu in the soil (Weber et al., 2009b; Hofacker et al., 2013). Manceau et al. (2008) observed metallic Cu at the soil-root interface of wetland plants.

1.4. Inorganic and organic sulfur biogeochemistry in wetland soils

The soil sulfur cycle plays a critical role for the biogeochemistry of trace metals in wetland soils. As it was outlined in the previous sections, sulfur is not only an important electron carrier (Ponnamperuma, 1972), but also strongly influences trace metal solubility by metal sulfide precipitation (Morse and Luther, 1999) or metal complexation by reduced organic sulfur groups (Smith et al., 2002; Karlsson et al., 2005; Skyllberg et al., 2006).

Total sulfur concentrations in wetland soils span a wide range from 60 to 5000 mmol kg\(^{-1}\) (Giblin and Wieder, 1992). In general, total sulfur concentrations in freshwater wetland soils are considerably lower than in marine or brackish wetland soils. For the riparian floodplain soils of the Mulde River (Germany) and the Dommel River (Netherlands), for example, total sulfur concentration of 80–240 mmol kg\(^{-1}\) have been reported (Poot et al., 2007). The total sulfur content of paddy soils in most Asian countries is often very low due to intensified cropping systems, leading to an enhanced output of nutrients, including S. The mean concentrations of total sulfur reported for paddy soils of a number of different countries range between 2.6 to 37 mmol kg\(^{-1}\) (Lefroy et al., 1992).

Sulfur occurs in soils in inorganic and organic forms in various oxidation states from -2 to +6 (Xia et al., 1998; Reddy and DeLaune, 2008; Prietzel et al., 2009). Inorganic sulfur comprises oxidized compounds, such as sulfate, sulfite and thiosulfate, and compounds of lower oxidation state, such elemental sulfur and sulfide. The organic sulfur fraction can be divided in two main groups (Stevenson, 1994; Reddy and DeLaune, 2008): Carbon-bonded sulfur comprises primarily thiols (R-SH) and organic mono- and disulfides (R-S-R', R-S-S-R') in S-containing peptides, proteins and amino acids (i.e. cystine, cysteine and methionine). Non-carbon-bonded sulfur primarily comprises ester sulfates (R-O-S) such as phenolic sulfates and sulfated polysaccharides.
1.4 Inorganic and organic sulfur biogeochemistry in wetland soils

<table>
<thead>
<tr>
<th>Sulfide</th>
<th>Mineral</th>
<th>pKs</th>
</tr>
</thead>
<tbody>
<tr>
<td>HgS</td>
<td>cinnabar</td>
<td>-39.5</td>
</tr>
<tr>
<td>CuS</td>
<td>covellite</td>
<td>-22.3</td>
</tr>
<tr>
<td>CdS</td>
<td>greenockite</td>
<td>-14.4</td>
</tr>
<tr>
<td>PbS</td>
<td>galena</td>
<td>-14.0</td>
</tr>
<tr>
<td>ZnS</td>
<td>sphalerite</td>
<td>-11.5</td>
</tr>
<tr>
<td>NiS</td>
<td>millerite</td>
<td>-5.6</td>
</tr>
<tr>
<td>FeS</td>
<td>mackinawite</td>
<td>-3.6</td>
</tr>
<tr>
<td>MnS</td>
<td>alabanite</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Under oxic conditions inorganic sulfur occurs mostly as adsorbed or precipitated sulfate, whereas metal sulfide minerals (e.g., pyrite FeS$_2$, mackinawite FeS) dominate sulfur speciation under permanently reducing conditions (Giblin and Wieder, 1992; Tabatabai, 2005). Low-pH conditions favor the adsorption of sulfate on Fe and Al (oxyhydro)oxides and clay minerals. In contrast, at pH >6 sulfate adsorption is negligible (Tabatabai, 2005). In freshwater wetland soils, most of the total soil sulfur (>90%) is present as organic sulfur, while in marine or brackish wetland soils reduced inorganic sulfur minerals can represent a larger proportion of the total sulfur (Giblin and Wieder, 1992). Carbon-bonded sulfur (reduced organic S) can account for up to 70–80% of the total sulfur content of NOM-rich freshwater wetland soils and humic substances (Wieder and Lang, 1988; Hutchison et al., 2001; Karlsson et al., 2005). In the microbial biomass, sulfur is predominantly present as reduced organic sulfur mainly as part of cysteine and methionine (Reddy and DeLaune, 2008). For a wide range of soils it has been shown that the microbial biomass sulfur fraction contains about 0.9–5.6% of the total soil organic sulfur (Banerjee and Chapman, 1996). This fraction is considered to be the most active pool for sulfur turnover in soils.

Sulfur is cycled between the various pools by four major transformation processes: mineralization, assimilation, reduction and oxidation (Siciliano and Germida, 2005). Mineralization is the metabolic conversion from organic sulfur into inorganic sulfur forms, which is mediated by various heterotrophic microorganisms and occurs under both aerobic and anaerobic conditions (Reddy and DeLaune, 2008). Depending on the microbial sulfur
requirements, mineralization is directly linked to assimilation (immobilization), when microorganisms incorporate released inorganic sulfur for the synthesis of cellular constituents. Otherwise sulfur is released as a byproduct during the metabolism of organo-sulfur compounds used as carbon or energy source. Both processes can occur simultaneously and generally result in a net immobilization at C:S ratios above 400 and a net mineralization at C:S ratios below 200 (Siciliano and Germida, 2005). Published mineralization rates suggest that sulfur mineralization is a relatively slow process. Zhou et al. (1999; 2005) found total sulfur mineralization in the range of 0.4−1.2 mmol kg$^{-1}$ and 0.5−1.1 mmol kg$^{-1}$ over 28 weeks of aerobic incubation of 12 upland soils and anaerobic incubation of 4 paddy soils, respectively.

Under strongly reducing conditions, obligate anaerobic sulfate reducing bacteria reduce sulfate as terminal electron acceptor to sulfide. For a riparian floodplain soil, an average sulfate reduction rate of 485 μmol kg$^{-1}$ d$^{-1}$ was determined at 23°C (Hofacker et al., 2013), which is significantly lower than rates reported for acid peatlands and salt marsh sediments (Reddy and DeLaune, 2008). The sulfate reduction rate in this soil was shown to decline with temperature, which significantly influenced trace metals sulfide formation during soil flooding. Other factors that can limit the rate of microbial sulfate reduction in wetland soils include low sulfate availability, a small community density of sulfate reducing bacteria, limited availability of respirable organic C and limited transport of dissolved substrates to particle-bound sulfate-reducing bacteria (Pallud and Van Cappellen, 2006). The oxidation of reduced sulfur compounds in the presence of O$_2$ can occur abiotically or can be catalyzed by chemolithotrophic bacteria, using O$_2$ and nitrate as electron acceptors (Reddy and DeLaune, 2008). The reduced organic sulfur fraction was found to be rather stable against oxidation, although redox transformations between thiol-S (-SH) and disulfide-S (-S-S-) cannot be excluded (Hutchison et al., 2001). Reduced inorganic sulfur compounds are in general labile in the presence of O$_2$. For example, during resuspension of a sulfide-rich sediment, 65 % of the initially present acid volatile sulfur (AVS) was oxidized within 2 hours, which was attributed to fast oxidation of FeS (Simpson et al., 1998). However, model studies showed that trace metal sulfides, like CdS, CuS, PbS, and ZnS were stable against oxidation over several hours (Simpson et al., 1998) and synthetic sulfide colloids of these metals persist in oxic waters even over 2 to more than 10 weeks (Sukola et al.,
Experiments with dredged sediments revealed that microorganisms significantly enhance the oxidation kinetics of trace metal sulfides under natural conditions (Lors et al., 2004). The susceptibility of trace metal sulfides to oxidative dissolution in natural systems is an important factor controlling the remobilization potential of individual trace metals during soil reoxidation.

**1.5. NOM as redox mediator and ligand for trace metals in wetland soils**

Natural organic matter (NOM) plays an important role for trace metal binding in soils (Tipping, 2002). Wetland soils which temporarily face reducing conditions are often characterized by high NOM contents as periods of high water contents can retard the decomposition of natural organic matter (Kirk, 2004). The major part of the natural organic matter in soils comprises humic substances, which contain various reactive functional groups for the binding of trace metal cations, such as carboxylic (-COOH), phenolic (-OH), amine (-NH₂) and sulfhydryl (-SH) groups (Stevenson, 1994). The majority of reactive sites in NOM below pH 7 are carboxylate moieties, which were shown to be important in binding of Cu, as Cu(II) forms very strong ring chelates by closely-spaced carboxyl groups and hydroxyl donors (Manceau and Matynia, 2010). However, for soft metal cations (e.g., Hg²⁺, Cd²⁺, Pb²⁺, Ag⁺, Cu⁺) (Parr and Pearson, 1983), complexation by amine and sulfhydryl groups as soft donor ligands can play an important role at low metal concentrations (Smith et al., 2002).

Using X-ray absorption spectroscopy, several studies over the last decade provided direct evidence for the binding of soft metal cations by reduced organic sulfur groups of humic and fulvic acids. For example, complexation by NOM thiol-groups has been reported for Cd²⁺ (Karlsson et al., 2005) and Hg²⁺ (Skyllberg et al., 2006), but has also been suggested for Zn²⁺ which is classified as an intermediate metal in the hard-soft metal concept (Karlsson and Skyllberg, 2007). Complexation of Cu by reduced organic sulfur groups have not been shown for terrestrial NOM so far. However, studies in marine and estuarine systems strongly suggest that thiol-containing organic ligands may play the key role in the stabilization of Cu⁺ against oxidation and disproportionation (Leal and van den Berg, 1998; Laglera and van den Berg, 2003). Reduction of Cu(II) to Cu(I) during flooding periods in wetland soils may thus enhance Cu
binding by reduced organic sulfur groups. Although the sulfur contents of soil humic substances are typically rather low (0.1−3.6 %; (Xia et al., 1998)), reduced organic sulfur groups may still play an important role for trace metal binding in wetland soils with low reducible sulfate content, as organic sulfur represents the dominant fraction of total sulfur (section 1.4). Additionally, the fraction of reactive organic sulfur sites can be enhanced under reducing condition by chemical addition of bisulfide (S$_2^-$) to NOM (Hoffmann et al., 2012).

NOM was also shown to play an important role as redox mediator for redox active trace metals. Humic substances contain a variety of redox active moieties, e.g., quinone-like functional groups, that cover a wide range of standard redox potentials between +0.1 and -0.3 V at pH 7 (Aeschbacher et al., 2010; Aeschbacher et al., 2011). NOM can therefore act as an electron donor and acceptor in the reductive transformation of redox active trace metals. The changing redox conditions in periodically flooded wetland soils can significantly influence the ability of NOM to act as electron donor or acceptor. Electrochemically reduced humic acid for example was shown to effectively reduce Hg(II) to volatile Hg(0) (Gu et al., 2011) and Ag(I) to Ag(0) nanoparticles (Maurer et al., 2012). In a recent study, it was reported that under anoxic conditions Cu(II) was reduced to Cu(I) even by untreated fulvic acid (Pham et al., 2012). However, Gu et al. (2011) also showed that strong complexation of Hg(II) by thiol functional or other reduced organic sulfur groups of NOM prevented the reduction of Hg(II) at low metal loadings. This shows that trace metal interactions with reduced humic acid involve a complex interplay between cation binding and electron transfer.

1.6. Research objectives and approach

The general aim of this PhD thesis was the investigation redox-controlled changes in Cu and Cd solubility and solid-phase speciation in periodically flooded soils. This project is divided in two major parts. The first part (chapters 2 and 3) addresses the effect of competitive metal sulfide formation on Cu and Cd dynamics during soil reduction and reoxidation. The second part (chapter 4) investigates redox and sorption interactions between Cu and natural organic matter. Specifically, the aim of the study presented in chapter 2 was to investigate how the amount of reducible sulfate affects Cu dynamics in the solution and solid phase during soil reduction and
1.6 Research objectives and approach

subsequent reoxidation. We hypothesized that in a Cu-Cd contaminated soil, Cu is the dominant sulfide forming metal. Depending on the initial amount of reducible sulfate, Cu will therefore either be reduced and precipitated as metal sulfide, or undergo different redox transformation reactions like reduction to Cu(0), when the amount of reducible sulfate is insufficient for complete Cu sequestration into metal sulfide. We further hypothesized that these variations in Cu speciation during soil reduction also affect Cu dynamics during subsequent soil reoxidation. To test these hypotheses, a time-resolved laboratory incubation experiment was conducted, in which an uncontaminated paddy soil from Bangladesh was spiked with Cu and Cd, adjusted to various metal-to-sulfate ratios and incubated over a reduction-reoxidation cycle. To characterize Cu solid-phase speciation and extractability, sequential extractions were combined with X-ray absorption spectroscopy. The temporal changes in Cu solubility and solid phase speciation over soil reduction and reoxidation are reported and discussed in relation to the initial amount of sulfate, dissolved sulfate dynamics and changes in reduced inorganic soil sulfur content.

The aim of the study presented in chapter 3 was to investigate how the amounts of reducible sulfate and the presence of Cu that competes with Cd for precipitation with biogenic sulfide influence solution and solid-phase dynamics of Cd over a reduction-reoxidation cycle. We hypothesized that depending on the initial amounts of sulfate and Cu in the soil, Cd will be either precipitated as sparingly soluble metal sulfide or will be outcompeted by Cu if the amount of reducible sulfate is insufficient to precipitate both metals. In the latter case, Cd will remain mobile during soil reduction and reoxidation. These hypotheses were tested within the same incubation experiment as outlined for chapter 2. Cd solid-phase speciation and extractability were studied by sequential extractions and X-ray absorption spectroscopy. The changes in Cd solubility and solid phase speciation are reported and discussed in relation to Cu dynamics described in chapter 2.

The aim of the study presented in chapter 4 was to investigate possible redox interactions between Cu and natural organic matter (NOM) under various redox conditions and related changes in Cu complexation. We hypothesized that electron transfer reactions between Cu and redox-active moieties of NOM may trigger Cu(I) and even Cu(0) formation. Based on the results of the first part of this thesis (chapter 2), we further hypothesized that reduction of Cu(II) to
Cu(I) significantly changes Cu binding by NOM, with reduced organic sulfur groups contributing to Cu(I)-NOM complexation. Redox transformations and complexation of Cu(II) and Cu(I) under both oxic and anoxic conditions were studied using untreated and electrochemically reduced soil humic acid (HA) as a model for redox-reactive NOM. Cu redox state and type of binding atoms were investigated by Cu K-edge X-ray absorption spectroscopy. This project part was complemented by a companion study (Maurer et al., 2013), which addressed Cu redox transformation and complexation by reduced and reoxidized HA, using potentiometric titrations and dialysis cell experiments. The aim of this companion study was to quantitatively determine Cu(I) binding to humic substances and to investigate kinetic aspects of Cu(I) formation in reduced and reoxidized HA solutions.

In chapter 5, the main findings from this PhD thesis are synthesized and discussed with respect to their application in the risk assessment of trace metal mobility and bioavailability in periodically flooded soils. Furthermore, future research needs are outlined.
1.7. References


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Lee, S. (2006) Geochemistry and partitioning of trace metals in paddy soils affected by metal mine tailings


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Maurer, F., Christl, I., Fulda, B., Voegelin, A. and Kretzschmar, R. (2013) Copper redox transformation and
complexation by reduced and oxidized soil humic acid: 2. Potentiometric titrations and dialysis cell


Morse, J.W. and Luther, G.W. (1999) Chemical influences on trace metal-sulfide interactions in anoxic


Cu(II), and Hg(II) by Fe(II)/Fe(III) hydroxysulfate green rust. *Chemosphere* **53**, 437-446.
1.7 References


2. Redox transformation, solid phase speciation and solution dynamics of copper during soil reduction and reoxidation as affected by sulfate availability

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**Abstract**

In periodically flooded soils, interactions of Cu with biogenic sulfide formed during soil reduction lead to the precipitation of sparingly soluble Cu-sulfides. In contaminated soils, however, the amounts of Cu can exceed the amount of sulfate available for microbial reduction to sulfide. In laboratory batch experiments, we incubated a paddy soil spiked to ~4.4 mmol/kg (280 mg kg\(^{-1}\)) Cu(II) to monitor temporal changes in the concentrations of dissolved Cu and the speciation of solid-phase Cu during 40 days of soil reduction and 28 days of reoxidation as a function of initially available reducible sulfate (0.06, 2.09 or 5.92 mmol kg\(^{-1}\)). Using Cu K-edge EXAFS spectroscopy, we found that a large fraction of Cu(II) became rapidly reduced to Cu(I) (23−39%) and Cu(0) (7−17%) before the onset of sulfate reduction. Combination with results from sequential Cu extraction and chromium reducible sulfur (CRS) data suggested that complexation of Cu(I) by reduced organic S groups (S\(_{\text{org}}\)) may be an important process during this early stage. In sulfate-depleted soil, Cu(0) and Cu(I)-S\(_{\text{org}}\) remained the dominant species over the entire reduction period, whereas in soils with sufficient sulfate, initially formed Cu(0) and (remaining) Cu(II) became transformed into Cu-sulfide during continuing sulfate reduction.
The formation of Cu(0), Cu(I)-S$_{org}$, and Cu-sulfide led to an effective decrease in dissolved Cu concentrations. Differences in Cu speciation at the end of soil reduction however affected the dynamics of Cu during reoxidation. Whereas Cu(0) was rapidly oxidized to Cu(II), more than half of the S-coordinated Cu fraction persisted over 14 days of aeration. Our results show that precipitation of Cu(0) and complexation of Cu(I) by reduced organic S groups are important processes in periodically flooded soils if sulfide formation is limited by the amount of available sulfate or the duration of soil flooding. The speciation changes of Cu described in this study may also affect the speciation and solubility of other chalcophile metals in redox-dynamic wetland soils.

2.1. Introduction

Copper (Cu) is an essential trace element for most living organisms. It is a constituent of a variety of proteins and enzymes that carry out fundamental biological functions (Rubino and Franz, 2012). However, elevated concentrations of Cu are toxic for microorganisms (Baath, 1989) and plants (Fernandes and Henriques, 1991). Ecosystem functioning can therefore be adversely affected as a result of soil contamination with Cu from anthropogenic activities such as metal mining or untreated waste water discharge.

While the behavior of Cu in well-drained oxic soils has been extensively studied, its biogeochemistry in periodically flooded soils, such as riparian wetlands and rice paddy soils, is less well-understood. In oxic soils, Cu dominantly occurs as bivalent (cupric) Cu(II). Cu(II) forms stable complexes with natural organic matter (Karlsson et al., 2006; Manceau and Matynia, 2010) and adsorbs on surfaces of Mn(III, IV) and Fe(III) (oxyhydr)oxides and clay minerals (Parkman et al., 1999; Strawn et al., 2004). However, Cu is a redox-active element and it may be reduced from cupric Cu(II) to cupreous Cu(I) or even Cu(0) by abiotic or biotic processes. Redox changes resulting from periodic overbank flooding and variations in groundwater level (Kirk, 2004) or flood irrigation (Koegel-Knabner et al., 2010) are therefore expected to strongly influence the chemical speciation and solubility of Cu in soils (Du Laing et al., 2009; Borch et al., 2010). Under sulfate-reducing conditions, the reduction of Cu(II) by dissolved sulfide (Luther et al., 2002; Ciglenecki et al., 2005) and subsequent precipitation as sparingly soluble metal
2.1 Introduction

Sulfides (Pattrick et al., 1997; Morse and Luther, 1999) may substantially decrease Cu solubility. Recent studies on trace metal dynamics in a contaminated river floodplain soil showed that even at low levels of available sulfide and in the presence of other chalcophile metals (e.g., Hg, Cd, Pb, Fe), Cu can be effectively sequestered as Cu sulfide, due to the high thermodynamic stability of Cu sulfide minerals (Weber et al., 2009a; Hofacker et al., 2013).

In general, total S contents in freshwater floodplain soils are considerably lower than in marine or brackish wetlands and comprise only a small fraction (< 5%) of inorganic S (Howarth et al., 1992). Riparian floodplain soils are aerated during most of the year, which prevents the accumulation of reduced inorganic S. Sulfate, on the other hand, is only weakly retained due to weak adsorption at pH > 6 (Tabatabai, 2005), and the mineralization of organic S is too slow to maintain high sulfate contents (Tabatabai and Alkhafaji, 1980; Zhou et al., 2005). As a result, the concentrations of chalcophile trace metals in contaminated freshwater floodplain soils may often exceed the amounts of reducible sulfate, resulting in the competition of different metal cations for reaction with limited amounts of sulfide formed by microbial sulfate reduction during periodic soil flooding (Weber et al., 2009a). Copper is expected to play a key role under sulfate-limited conditions, because it forms much more stable metal sulfides than Fe or trace metals like Pb, Cd, or Zn and typically occurs at much higher concentrations than Hg or Ag that would form even less soluble sulfides than Cu. However, in addition to the thermodynamic stability of metal sulfides, the solubility of other metal bearing phases and the kinetics of metal desorption, diffusion, and precipitation are also expected to strongly influence the formation of metal sulfide minerals (Weber et al., 2009a).

At low sulfide levels, Cu(II) may also be reduced by microorganisms (Wakatsuki, 1995), by reaction with dissolved Fe(II) (Matocha et al., 2005), or by redox-active functional groups of natural organic matter as recently shown (Pham et al., 2012). Biotic or abiotic reduction of Cu(II) to Cu(I) in sulfate-limited environments may have two major effects: The soft metal cation Cu⁺ may form strong complexes with thiol-groups of natural organic matter (R-SH), as reported for other soft metal cations like Ag⁺, Cd²⁺ and Hg²⁺ (Smith et al., 2002; Karlsson et al., 2005; Skyllberg et al., 2006). Association of Cu(I) with thiol-containing ligands has long been known to play a role in marine environments (Boulegue et al., 1982; Leal and van den Berg, 1998), but has...
hardly been considered for terrestrial soils. Another consequence of Cu reduction in sulfate-limited environments may be the formation of metallic Cu(0), since Cu(I) can rapidly disproportionate to Cu(II) and Cu(0) if the concentrations of Cu(I)-stabilizing ligands (e.g., sulfide, chloride, thiol) are too low. Metallic Cu has been observed in and near roots of wetland plants (Manceau et al., 2008) and in organic-rich bog soils (Lovering, 1927; Lett and Fletcher, 1980). Recently, Cu(0) formation has also been observed in microcosm flooding experiments with a contaminated floodplain soil (Weber et al., 2009b; Hofacker et al., 2013). In these studies Cu(0) was formed as intermediate phase, which was completely sulfidized during subsequent sulfate reduction. Under sulfate-limited conditions, however, Cu(0) may accumulate in reduced soils.

Variations in Cu speciation changes during soil reduction may also affect Cu dynamics during subsequent soil reoxidation, because different reduced Cu species may vary with respect to their reactivity under oxic conditions. While Cu sulfide clusters and Cu(I)-thiol complexes were shown to be stable over days to weeks in oxic waters (Leal and van den Berg, 1998; Rozan et al., 2000; Laglera and van den Berg, 2003; Sukola et al., 2005), Cu(0) oxidation occurs within tens of minutes (Kanninen et al., 2008).

Knowledge on the behavior of Cu during soil reduction and reoxidation at low sulfate availability is very limited (Weber et al., 2009a), and studies on Cu mobilization during transition from anoxic to oxic conditions were mainly conducted with sulfide-rich sediments and soils, focusing on the oxidative dissolution of Cu sulfides (e.g., Simpson et al., 1998; Carroll et al., 2002; Caetano et al., 2003). Thus, the aim of the present study was to elucidate the effect of sulfate availability on Cu dynamics in solution and solid phases during soil reduction and subsequent reoxidation.

We conducted a time-resolved laboratory incubation experiment, in which we adjusted an uncontaminated paddy soil from Bangladesh to various sulfate-to-Cu ratios prior to an anoxic incubation period of 40 days, followed by reoxidation for 28 days. The dynamics of Cu in the solution phase and changes in Cu solid phase speciation were investigated by Cu K-edge X-ray absorption spectroscopy (XAS), sequential metal extractions, and chromium-reducible sulfur (CRS) distillation.
2.2. **Materials and Methods**

2.2.1. **Soil sampling and characterization**

A large batch of topsoil (∼150 kg, 0–10 cm depth) was collected from a rice paddy field near Sreenagar (Munshiganj district, Bangladesh) after the rice harvest in May 2007. The soil was classified as a non-calcareous Hydragric Anthrosol (Hypereutric, Siltic) with a silty clay loam texture (Dittmar et al., 2007).

The soil material was oven-dried at 60 °C, broken up into soil aggregates < 2 cm using a jaw crusher (Retsch, Germany), mixed homogenously, and stored in plastic containers in the dark. Physical and chemical soil properties were determined by soil chemical standard methods. Soil pH was measured in 10 mM CaCl$_2$ solution. Soil C and N contents were measured using a CHNS Analyzer (CHNS-932; LECO, USA). Total contents of other major elements (Z>11), including trace metals and S, were determined by energy dispersive X-ray fluorescence spectrometry (XRF; X-Lab 2000; Spectro, Germany). The total amount of reducible sulfate was estimated by a three-fold 0.5 M NaHCO$_3$ extraction at pH 8.5 (adjusted with NaOH) (Kilmer and Nearpass, 1960). The extracted sulfate was measured by ion chromatography (IC) with suppressed conductivity detection (Metrosep A Supp. 5 column; Metrohm, Switzerland). The amount of reduced inorganic S was determined by chromium(II)-reducible sulfur extraction (CRS, see section 2.2.4 for details) (Canfield et al., 1986).

Selected physical and chemical soil properties are summarized in Table 2.1. The soil had a weakly acidic pH of 6.2 and contained 2.3% organic carbon and 33.4% clay. The trace element concentrations were low and within the typical range of background values reported for Bangladesh paddy soils (Ali et al., 2003). The total S content (12.6 mmol kg$^{-1}$) was also in line with values reported for freshwater wetland soils (Lefroy et al., 1992). The amount of sulfate available for anaerobic microbial respiration was 2.09 mmol kg$^{-1}$. Of the total S, 0.95 mmol kg$^{-1}$ was present as reduced inorganic S (CRS$_{\text{bkg}}$), probably mainly in recalcitrant sulfide minerals like pyrite (FeS$_2$) that are stable against oxidation. The remaining S was most likely bound in soil organic matter (SOM).
Table 2.1. Selected physical and chemical characteristics of the untreated soil used in the experiment (mean ± standard deviation (SD); n=10 unless stated otherwise).

<table>
<thead>
<tr>
<th>Soil property</th>
<th>Units</th>
<th>Mean</th>
<th>SD</th>
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<tr>
<td>pH</td>
<td>--</td>
<td>6.23</td>
<td>± 0.03</td>
</tr>
<tr>
<td>clay</td>
<td>(g kg(^{-1}))</td>
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</tr>
<tr>
<td>C(_{\text{anorg}})(^{b})</td>
<td>(g kg(^{-1}))</td>
<td>&lt; 0.2</td>
<td></td>
</tr>
<tr>
<td>C(_{\text{org}})(^{c})</td>
<td>(g kg(^{-1}))</td>
<td>22.7</td>
<td>± 1.1</td>
</tr>
<tr>
<td>N(_{\text{tot}})(^{c})</td>
<td>(mmol kg(^{-1}))</td>
<td>171</td>
<td>± 3</td>
</tr>
<tr>
<td>S(_{\text{tot}})</td>
<td>(mmol kg(^{-1}))</td>
<td>12.6</td>
<td>± 0.3</td>
</tr>
<tr>
<td>SO(_{4}^{2-})(^{d})</td>
<td>(mmol kg(^{-1}))</td>
<td>2.09</td>
<td>± 0.01</td>
</tr>
<tr>
<td>CRS(_{\text{dkg}})(^{e})</td>
<td>(mmol kg(^{-1}))</td>
<td>0.95</td>
<td>± 0.06</td>
</tr>
<tr>
<td>Fe</td>
<td>(mmol kg(^{-1}))</td>
<td>866</td>
<td>± 5</td>
</tr>
<tr>
<td>Mn</td>
<td>(mmol kg(^{-1}))</td>
<td>10.0</td>
<td>± 0.1</td>
</tr>
<tr>
<td>Cu</td>
<td>(mmol kg(^{-1}))</td>
<td>0.92</td>
<td>± 0.03</td>
</tr>
<tr>
<td>Cd</td>
<td>(mmol kg(^{-1}))</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>(mmol kg(^{-1}))</td>
<td>0.37</td>
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</tr>
<tr>
<td>Zn</td>
<td>(mmol kg(^{-1}))</td>
<td>1.75</td>
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</tr>
<tr>
<td>Ni</td>
<td>(mmol kg(^{-1}))</td>
<td>1.15</td>
<td>± 0.03</td>
</tr>
<tr>
<td>As</td>
<td>(mmol kg(^{-1}))</td>
<td>0.21</td>
<td>± 0.01</td>
</tr>
</tbody>
</table>

\(^{a}\) major element contents were measured by XRF unless stated otherwise; \(^{b}\) Dittmar et al. (2007); \(^{c}\) determined by CHNS analyzer; \(^{d}\) NaHCO\(_3\) extractable sulfate (n=3); \(^{e}\) reduced inorganic S determined by CRS distillation (n=4).

2.2.2. Soil incubation experiment

For the incubation experiment the soil material was gently crushed with a pestle to decrease soil aggregate size to < 2 mm and coarse organic material (roots, straw) was removed by hand. Five different incubation series were conducted in which the soil was adjusted to different Cu and sulfate concentrations as summarized in Table 2.2. In one series the soil was washed to remove exchangeable sulfate and subsequently spiked with CuCl\(_2\) solution ("low-sulfate" series, LS). In two other series, the soil was used as is and spiked with either only CuCl\(_2\) ("medium-sulfate" series, MS) or a mixed CuCl\(_2\) / CaSO\(_4\) solution ("high-sulfate" series, HS). Additionally, two control series without CuCl\(_2\) addition were conducted for the LS-series ("low-sulfate control" series, LSC) and the MS-series ("medium-sulfate control" series, MSC), and processed in the same way as the metal-spiked series. All solutions were adjusted with CaCl\(_2\) to a total
2.2 Materials and Methods

Table 2.2. Total Cu and sulfate in the spiked soils. The untreated soil contained 0.92 mmol kg\(^{-1}\) Cu and 2.09 mmol kg\(^{-1}\) extractable sulfate (Table 2.1).

<table>
<thead>
<tr>
<th>Incubation series</th>
<th>Abbreviation</th>
<th>Cu (mmol kg(^{-1}))(^a)</th>
<th>Sulfate (mmol kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>low sulfate</td>
<td>LS</td>
<td>4.42 ± 0.05</td>
<td>0.06 ± 0.03(^b)</td>
</tr>
<tr>
<td>medium sulfate</td>
<td>MS</td>
<td>4.43 ± 0.07</td>
<td>2.09 ± 0.01</td>
</tr>
<tr>
<td>high sulfate</td>
<td>HS</td>
<td>4.44 ± 0.10</td>
<td>5.92 ± 0.04</td>
</tr>
<tr>
<td>low sulfate control</td>
<td>LSC</td>
<td>0.92 ± 0.03</td>
<td>0.06 ± 0.03(^b)</td>
</tr>
<tr>
<td>medium sulfate control</td>
<td>MSC</td>
<td>0.91 ± 0.03</td>
<td>2.09 ± 0.01</td>
</tr>
</tbody>
</table>

\(^{a}\) Total Cu concentration after incubation determined by XRF (mean ± standard deviation; n=11); \(^{b}\) NaHCO\(_3\) extractable sulfate minus sulfate extracted during soil washing (uncertainty from Gaussian error propagation).

The background concentration of 5 mM Cl\(^-\). Note that in all Cu-spiked treatments, the soil was also spiked with 0.2 mmol kg\(^{-1}\) Cd using CdCl\(_2\) to determine the coupled effects of Cu and varying sulfate content on Cd transformation during soil reduction and reoxidation. The results for Cd will be reported in a separate publication. The spiked amount of Cd resulted in a low molar Cd/Cu ratio of 0.045 at which Cd was not expected to significantly affect Cu transformation processes investigated in the present study.

For soil incubation, 20 g of dry soil was filled into 120 mL crimp vials (three replicates per sampling time) and suspended in 40 mL of the respective metal spike or background solution. For the LS and LSC series, the soil was washed three times for 1 h with 80 mL of 1 mM CaCl\(_2\) solution before the addition of metal spike or background solution. After each washing step the suspensions were centrifuged (15 min at 1164 \(g\)) and the supernatants were discharged. Aliquots of the supernatants were taken from 9 vials after each washing step, respectively, and analyzed for extracted sulfate (IC), dissolved organic carbon (TOC-5000; Shimadzu, Japan), and major cations by inductively-coupled plasma optical emission spectrometry (ICP-OES; Vista MPX; Varian, USA). During the soil washing procedure, 2.02±0.03 mmol kg\(^{-1}\) sulfate (mean ± standard deviation, n=9) were removed from the soil. According to the total exchangeable amount of sulfate in the untreated soil, determined by NaHCO\(_3\) extraction (2.09 mmol kg\(^{-1}\)), the residual amount of sulfate available for anaerobic respiration in the low-sulfate series was then 0.06±0.03 mmol kg\(^{-1}\) (Table 2.2). The fraction of major cations and trace elements removed during soil washing was negligible (< 1% of the respective total element content). After the last
washing step the wet soil paste contained 16 mL of solution. Therefore, 24 mL of a more concentrated metal spike or background solution was added to adjust the solid-to-solution ratio to 0.5 g mL$^{-1}$ as in the other incubation series.

The soil reduction-reoxidation cycle was induced as follows: After addition of the metal spike or background solution, the incubation vials were pre-equilibrated for 2 days on a horizontal shaker in contact with air ("equilibration phase", EQ; preliminary experiments showed that stable dissolved Cu concentrations were reached after equilibration for 2 days). After equilibration, a supplementary C source (5 mM Na-lactate) and 2.5 mL soil suspension (~1 g soil dry weight in 2 mL) containing the natural microbial community were added as inoculum to stimulate microbial activity. The inoculum was prepared analogously to the 2-day equilibrated samples of the respective control series. The vials were subsequently sealed with a butyl rubber septum and, after replacing the headspace with nitrogen gas, incubated anoxically for up to 40 days at 28.0±1.2 °C with continuous end-over-end shaking ("reduction phase", RED). Subsequently, the vials were opened and allowed to reoxidize for up to 4 weeks by horizontal shaking in contact with air ("reoxidation phase", REOX).

### 2.2.3. Sampling and analysis of solution and gas phases

From each incubation series three incubation vials per sampling time were used for gas, solution, and solid phase analyses. The samples were taken after the EQ-phase and at different time points within the RED-phase (day 1, 5, 10, 15, 20, 30, and 40), and the REOX-phase (day 2, 7, 14, and 28), respectively. CO$_2$ partial pressure was measured in the headspace by gas chromatography (GC 8610C, SRI Instruments, Germany) using a flame ionization detector and a CO$_2$ methanizer. Dissolved inorganic carbon (DIC) was calculated from headspace concentrations using Henry's law and carbonate equilibrium constants (Appendix A.2). After gas sampling, all further sample processing was done in a glovebox (Braun, Germany) under N$_2$ atmosphere (O$_2$ < 1 ppm). The soil suspensions were transferred into centrifuge vials and centrifuged (10 min at 3566 $g$). The supernatants were decanted and filtered through 0.2 μm nylon filters (Opti-Flow; Wicom, Germany). Solution pH and E$_h$ were measured in the unfiltered supernatants. Aliquots of the filtered supernatants were used for the determination of sulfate.
and other major anions (IC). Further aliquots were acidified (1% v/v of concentrated HCl) for the determination of dissolved organic carbon (TOC analyzer), major cations (ICP-OES), and Fe(II) by UV-vis spectrometry (Cary 50 Bio, Varian) using the 1,10-phenanthroline method after Tamura et al. (1974).

### 2.2.4. Sampling and analysis of solid phase

The soil material from the three replicates per sampling time was combined, homogenized, divided into subsamples, sealed in gas tight bags and frozen in liquid nitrogen. For sequential metal extractions a subsample of the frozen soil was allowed to thaw in the glovebox and used immediately as wet paste to prevent potential artifacts induced by rewetting of the freeze-dried samples (Hjorth, 2004). Another subsample was freeze-dried for determination of chromium(II)-reducible sulfur (CRS), Cu speciation by X-ray absorption spectroscopy (XAS), and analysis of total elemental composition by XRF. The freeze-dryer was purged with Ar at the beginning and the end to minimize sample contact with \( \text{O}_2 \) during freeze-drying. The dried samples were stored in the \( \text{O}_2 \)-free glovebox until analysis. We previously obtained similar CRS results for wet and freeze-dried soil samples (Weber et al., 2009). With respect to XAS analysis, we considered freeze-drying to negligibly affect metal speciation as long as the samples were kept anoxic and dry until analysis.

Using selected soil samples (2d EQ, 10d RED, 40d RED, 28d REOX) from the metal-spiked series (LS, MS, HS), changes in metal partitioning over the reduction-reoxidation cycle were determined by a five-step sequential extractions procedure on wet soil pastes as summarized in Table 2.3. The first three extraction steps were conducted in the glovebox using deoxygenated solutions to prevent sample oxidation (Rapin et al., 1986). Extractions were performed in triplicate using about 1 g of soil per replicate (dry-weight basis). After each extraction step, the samples were centrifuged (15 min at 3566 \( g \)) and the soil residuum was washed once to prevent carry-over of residual solution into the next step. Extracts were filtered through 0.45 μm nylon filters (Opti-Flow; Wicom, Germany), acidified (1% v/v concentrated HCl), and analyzed by ICP-OES using multi-element standards with the matrix of the respective extract.
Table 2.3. Summary of the five-step sequential extraction procedure and hypothetical interpretation of the five fractions. The residual fraction (RES) was calculated as the difference between total contents determined by XRF analysis and the sum of the five extractions steps. The solid-to-solution ratio (SSR) is given in g mL⁻¹. Steps F1 to F3 were conducted under N₂ atmosphere (O₂ < 1 ppm).

<table>
<thead>
<tr>
<th>Step</th>
<th>Extraction solution</th>
<th>Extraction conditions</th>
<th>Hypothetical interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1 a</td>
<td>0.1 M CaCl₂</td>
<td>24 h shaking; 23 °C; SSR 1:25</td>
<td>mobile fraction (soluble and exchangeable, soluble metal-organic complexes)</td>
</tr>
<tr>
<td>F2 b</td>
<td>1 M NaOAc (pH 5)</td>
<td>24 h shaking; 23 °C; SSR 1:25</td>
<td>easily mobilizable fraction (specifically adsorbed, bound to CaCO₃ and other minerals labile at pH 5, weak metal-organic complexes)</td>
</tr>
<tr>
<td>F3 c</td>
<td>0.025 M NH₄-EDTA (pH 4.6)</td>
<td>90 min shaking; 23 °C; SSR 1:25</td>
<td>organically bound fraction (low affinity sites)</td>
</tr>
<tr>
<td></td>
<td>1 M NH₄OAc (pH 4.6)</td>
<td>10 min shaking; 23 °C; SSR 1:12.5</td>
<td></td>
</tr>
<tr>
<td>F4 c</td>
<td>0.1 M ascorbic acid + 0.2 M NH₄Ox/HOx (pH 3.25)</td>
<td>2 h; 96 °C (heat block); SSR 1:25</td>
<td>reducible fraction (bound to amorphous and crystalline Fe- and Mn-oxides)</td>
</tr>
<tr>
<td></td>
<td>0.2 M NH₄Ox/HOx (pH 3.25)</td>
<td>10 min shaking; 23 °C; SSR 1:12.5</td>
<td></td>
</tr>
<tr>
<td>F5 b</td>
<td>30% H₂O₂ (pH 2) + 2 M HNO₃</td>
<td>5 mL H₂O₂ + 3 mL HNO₃; 2 h; 85 °C (heat block) + 3 mL H₂O₂; 3 h; 85 °C (heat block)</td>
<td>oxidizable fraction (metal sulfides and organically bound fraction (high affinity sites))</td>
</tr>
<tr>
<td></td>
<td>3.2 M NH₄OAc (in 20% v/v HNO₃)</td>
<td>+ 5 mL NH₄OAc; 30 min; 23°C</td>
<td></td>
</tr>
</tbody>
</table>

a McGrath and Cegarra (1992); b Tessier at al. (1979); c Zeien and Brümmer (1989); d After steps F1 to F4, soil residuum was washed with doubly deionized water for 10 min (SSR 1:12.5 for F1, F2, F4, 1:25 for F3).
Changes in the amounts of reduced inorganic S over the reduction-reoxidation cycle were determined by chromium(II)-reducible sulfur extraction (CRS) adapting the distillation method of Canfield et al. (1986). 10 g of freeze-dried soil were weighed into a round-bottom flask and connected to the distillation apparatus that was immediately purged with N\textsubscript{2} gas. 20 mL of concentrated HCl and 40 mL of 1 M CrCl\textsubscript{2} in 0.5 M HCl (prepared by reaction of 1 M CrCl\textsubscript{3} in 0.5 M HCl with Zn granules; (Burton et al., 2008) was injected and heated for 2 h under continuous N\textsubscript{2} gas flow. Liberated H\textsubscript{2}S was trapped in a 0.1 M Zn-acetate solution and determined by iodometric titration. The recovery of sulfide liberated by the CRS extraction of CuS (Alfa Aesar, 042925; predominantly covellite based on XRD) in different matrices was tested by spiking 10 g of quartz sand or oxic soil to a sulfide content of about 6 or 30 mmol kg\textsuperscript{-1}. The S recovery was independent of the spiked amount of CuS and reached 87±2 % in the quartz matrix and 76±1 % in the oxic soil matrix, possibly due to side reactions of released sulfide with soil constituents.

2.2.5. Cu K-edge X-ray absorption spectroscopy

Cu speciation in selected soil samples (2d EQ, 10d RED, 40d RED, 14d REOX) from the metal spiked series (LS, MS, HS) were analyzed by Cu K-edge X-ray absorption spectroscopy (XAS). X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine-structure (EXAFS) spectra were recorded at the beamlines XAS and INE at the Angströmquelle Karlsruhe (ANKA, Karlsruhe, Germany). At both beamlines, the double crystal Si(111) monochromator was calibrated by setting the first inflection of the K-edge of a metallic Cu foil to 8979 eV. The monochromator was detuned to 65% of its maximal intensity to reduce higher harmonics in the beam. Spectra of soil samples were recorded in fluorescence mode using a 5-element Ge solid state fluorescence detector (average of 4–6 scans with 1 h measurement time each). Spectra of Cu reference compounds were recorded in transmission mode. To account for small energy shifts, a metallic Cu foil was measured regularly between the scans. Freeze-dried soil samples were ground, pressed into pellets and placed between Kapton\textsuperscript{®} tape under dry N\textsubscript{2} atmosphere and kept anoxic until the measurement. All samples and most references were measured at the INE beamline at 77 K using a liquid N\textsubscript{2} cryostat. Reference spectra measured at the XAS beamline were recorded at 15 K using a closed-cycle He-cryostat. Additional reference spectra published
in the literature were kindly provided by other authors. The sources of all reference spectra are
given in Appendix A (Table A.1).

All spectra were processed using the analysis program Athena (Ravel and Newville, 2005). For
data extraction, $E_0$ was fixed to 8988 eV. Background correction was performed by subtracting a
first-order polynomial fit to the pre-edge region (−120 to −40 eV) and subsequently dividing by
a second-order polynomial fit to the post-edge region (80 to 530 eV). The background spline was
adjusted using the Autobk algorithm ($R_{bkg} = 0.9$; $k$-weight = 3; $k$-range = 0.5–11.4 Å$^{-1}$). EXAFS
data were Fourier transformed over the $k$-range 2.5–10 Å$^{-1}$ using a Kaiser-Bessel apodization
window (sill width = 2.5 Å$^{-1}$).

Soil spectra were analyzed by linear combination fitting (LCF) of the $k^3$-weighted EXAFS data
over the $k$-range 2.5 to 10 Å$^{-1}$. The sum of all fitted fractions was not constrained, but individual
fractions were constrained to range between 0 and 1. A variety of spectra of organic and
inorganic Cu(II) and Cu(I) reference compounds with different coordination geometries, first
shell atoms (O/N and/or S) and distances as well as metallic Cu were considered for LCF (see
Appendix A Table A.1). Based on principal component analysis (PCA) and target transform
testing (TT) (for details see Appendix A.1), the reference spectra of metallic Cu foil (Cu metal)
and of Cu(II) adsorbed to montmorillonite (Cu(II)-clay; proxy for O-coordinated Cu(II)) and
freshly-precipitated primitive copper sulfide (Cu$_{32}$S$_{prim}$; proxy for S-coordinated Cu(I); spectrum
"2n – blue/black primitive" from Pattrick et al. (1997), kindly provided by Fred Mosselmans,
Diamond Light Source, Didcot, UK) were selected for three-component LCF analysis. For an
additional four-component LCF analysis, one of the following organic Cu(I)-S complexes was
included: (1) Cu(I) diethylthiocarbamate (Cu(I)-carbamate); (2) Cu(I) glutathione (Cu(I)-GSH);
(3) bis(tetraphenylphosphonium) tris(thiophenolato)cuprate(1) [(C$_6$H$_5$)$_3$P]$_2$Cu(SC$_6$H$_5$)$_3$ (Cu(I)-S-
trigonal; spectrum "Cu(I)-3S" from Pufahl et al. (1997), kindly provided by James Penner-Hahn,
University of Michigan); (4) Cu(I) coordinated by 1N (His) and 2-3S (Met) in the copper
trafficking protein CopC (Cu(I)-CopC; spectrum form Arnesano et al. (2003), kindly provided by
Stefano Mangani, Università di Siena).
2.3. Results

2.3.1. Solution dynamics during soil reduction and reoxidation

The dynamics of $E_h$, pH, DIC, DOC, major cations and anions, and Cu in solution during the reduction-reoxidation cycle followed similar trends in all incubation series (Figure 2.1 and Appendix A Figures A.3 and A.4). The observed changes in dissolved concentrations can be explained by the microbial respiration of electron acceptors following the classical sequence of redox reactions observed in submerged soils (Kirk, 2004; Koegel-Knabner et al., 2010). In the metal-spiked series (LS, MS, HS), soil reduction was slightly retarded compared to the two control series (LSC, MSC), probably due to a toxic effect of the metal spike that suppressed soil microbial activity (Baath, 1989; Jin et al., 2007). However, the three metal-spiked series contained the same amount of Cu and Cd and were therefore comparable with respect to overall microbial soil redox dynamics. In the following, we will explain the trends observed during the reduction (RED) and reoxidation (REOX) phases in more detail.

Reduction phase (RED)

Upon exclusion of $O_2$, the $E_h$ dropped rapidly and then stabilized between 0 and $-150$ mV (Figure 2.1a) and the pH gradually increased from 6.5 to 7.1 (Figure 2.1b). The pH increase is a result of proton-consuming reduction processes such as the reductive dissolution of Mn(III, IV) and Fe(III) (oxyhydr)oxides (in the following referred to as Mn- and Fe-oxides). A fast initial increase in DIC indicated a rapid microbial consumption of organic carbon substrates within the first 15 days (Appendix A Figure A.4a). Dissolved organic carbon (DOC) increased with the addition of lactate but showed no further significant changes during anoxic incubation in the metal-spiked series (Figure 2.1c). This implies a release of DOC from the soil, possibly induced by the accumulation of organic metabolites (acetate and propionate), the increasing pH, and liberation of DOC by dissolution of Fe- and Mn-oxide mineral phases (Grybos et al., 2009). The carbon turnover in the control series was faster and counteracted DOC release, resulting in a gradual decrease of DOC.
Figure 2.1. Solution dynamics during soil reduction and reoxidation for the Cu-spiked (left panels) and control series (right panel). Error bars indicate the standard deviation of experimental triplicates. Dashed arrows in panel e) indicate the start of slow initial sulfate reduction, solid arrows the start of fast sulfate reduction.
The microbial respiration of terminal electron acceptors in the absence of $O_2$ followed the typical order of nitrate, Mn- and Fe-oxides, and sulfate. Nitrate was already consumed within one day (Appendix A Figure A.3a). The reduction of Mn and Fe started within 1 to 5 days after $O_2$ exclusion and resulted in the increase of dissolved Mn (Appendix A Figure A.3b) and Fe (Figure 2.1d; predominantly Fe(II)). Dissolved Ca and Mg concentrations tripled during the reduction period (Appendix A Figure A.3d and e), which can be attributed to cation exchange with dissolved Fe and Mn (Zachara et al., 2001; Weber et al., 2009a).

About 95% of the total extractable sulfate was found in solution after 5 days of reduction (Figure 2.1e). The remaining sulfate was most likely adsorbed to Fe- and Al-oxides and clay minerals (Chao et al., 1962). The adsorption of sulfate is strongly pH-dependent and considered to be negligible at pH > 6.5 (Curtin and Syers, 1990), which explains the high fraction of dissolved sulfate. The maximal dissolved sulfate concentrations in the low-sulfate treatments (LCS and LS) after 1 and 10 days of reduction indicated the presence of about 0.10 and 0.13 mmol kg$^{-1}$ sulfate available for sulfate reducing bacteria in these series. These amounts were slightly higher than the amount of residual sulfate after soil washing (Table 2.2). The latter value was calculated by subtracting sulfate in the washing solution from the total extractable sulfate of the untreated soil and was associated with substantial analytical uncertainty. Therefore, in all further calculations for the low-sulfate treatments, the upper limit of initially available sulfate was derived from maximal dissolved sulfate concentrations which were considered more reliable as they could be directly measured.

In the MS and HS series, at most 0.2 and 0.4 mmol kg$^{-1}$ sulfate were consumed during slow initial sulfate reduction from day 5 up to day 15, as calculated by the difference in dissolved sulfate concentrations. The main period of sulfate reduction, reflected by a significant decrease of dissolved sulfate with time, started at day 1 (LSC, MSC), day 10 (LS), and day 15 (MS, HS), respectively. Available sulfate was entirely consumed until day 5 (LSC), day 15 (MSC), and day 30 (LS, MS, HS), respectively. The sulfate reduction rates (LSC and LS 6−25 μmol kg$^{-1}$ d$^{-1}$; MSC and MS 119−139 μmol kg$^{-1}$ d$^{-1}$; HS 335 μmol kg$^{-1}$ d$^{-1}$) were linearly correlated with the initial amount of extractable sulfate ($R^2 = 0.992$), suggesting that microbial sulfate reduction was not substrate-limited and that the metal spike mainly influenced the onset of sulfate reduction.
rather than its rate (Appendix A Figure A.5a). Throughout the reduction phase, about 0.1–0.2 mmol L\(^{-1}\) unspecified (non-sulfate) S was found in solution, probably representing organic S in DOM (Appendix A Figure A.3c).

After the 2-day pre-equilibration phase (EQ), less than 0.1% of the spiked Cu remained dissolved (Figure 2.1f). The sharp increase in Cu concentrations to 4–8 µM within the first day of the reduction phase (RED) in the spiked treatments was attributed to the addition of 5 mM lactate. This probably led to a short-term disequilibrium due to complexation of small amounts of weakly adsorbed Cu or mobilization of adsorbed Cu-organic matter complexes. In the two control series, dissolved Cu did not increase upon lactate addition, suggesting that spiked Cu was more readily mobilizable than native soil Cu. After day 1 of the reduction phase (RED), dissolved Cu decreased rapidly and fell below the detection limit (0.05 µM) after day 40 (LS), day 30 (MS), and day 15 (HS), respectively.

Reoxidation phase (REOX)

Upon aeration of the soil suspensions, the E\(_a\) increased from −100 mV to 300 mV within two days, followed by a slow further increase for 4 weeks to values close to those measured before soil reduction (Figure 2.1). Concurrently, Mn and Fe were removed from solution within two days, which we attributed to oxidation of Fe(II) and Mn(II) and subsequent precipitation of Fe- and Mn as (oxyhydr)oxides. Solution pH also increased rapidly to about 8.4, due to release of CO\(_2\) from accumulated bicarbonate, and then decreased again approaching similar values as before soil reduction. The acidity produced by oxidative processes and hydrolysis of Fe(III) and Mn(III, IV) was effectively buffered by dissolved or solid carbonate species.

During soil reoxidation, dissolved sulfate increased to concentrations similar to (MS, HS) or even higher (LS, LSC, MSC) than those measured prior to soil reduction. The higher sulfate concentrations pointed towards mineralization of organic S over the course of soil incubation. The close correlation between initial sulfate release rates during reoxidation and initially available soil sulfate and the observation that total inorganic S increased mainly during soil reoxidation suggested that mineralization of organic S did not significantly contribute to sulfide formation during soil reduction (for details see Appendix A.3), in line with the low rate of
S mineralization reported for flooded paddy soils (Zhou et al., 2005). In the HS and MSC series, sulfate release was accompanied by a sharp rise in unspecified S followed by a steady decline towards the end of the reoxidation phase (Appendix A Figure A.3c). This probably reflected the formation of inorganic S species such as thiosulfate and elemental S as intermediates during the oxidation of sulfide to sulfate (Burton et al., 2006).

Upon aeration, dissolved Cu concentrations in all series increased immediately to 2- to 5-fold higher values than after the equilibration period. For the LS series the initial increase was slightly steeper. Dissolved Cu remained high over two weeks of reoxidation in all series and subsequently declined concomitant to pH and DOC concentrations.

2.3.2. Solid-phase S dynamics during soil reduction and reoxidation

Figure 2.2a shows the development of chromium-reducible sulfur (CRS) contents of selected soil samples during the reduction-reoxidation cycle. The difference between the CRS content of the samples and the background value of the oxic soil (CRS_{bkg}) can be interpreted as the CRS newly formed during sulfate reduction (CRS_{add} = CRS - CRS_{bkg}). During the first 10 days of reduction, there was only a minor increase in CRS_{add}, which was in line with the observed changes in dissolved sulfate (Figure 2.1e). After 40 days of reduction, 2.1 and 4.6 mmol kg\(^{-1}\) CRS_{add} was formed in the MS and HS treatments. In the low-sulfate treatments, CRS_{add} contents remained low even at the end of the reduction period (CRS_{add} 0.4–0.5 mmol kg\(^{-1}\)). A significant fraction of the CRS_{add} was stable over 28 days of soil reoxidation (40% in MS and 30% in HS).

In Figure 2.2b, CRS_{add} values are compared with the potential sulfide formation, which was calculated as the difference between the total amount of initially available sulfate and dissolved sulfate concentration at each time point. The CRS_{add} contents in the metal-spiked series after 10 and 40 days of reduction correlated very well with potential sulfide formation (R\(^2\) = 0.99). However, the CRS_{add} contents were lower than the potential sulfide formation, suggesting that sulfide formation was underestimated by the CRS analysis, most likely due to incomplete extraction. The slope of the correlation between CRS_{add} and potential sulfide formation (0.76) exactly matched the recovery determined in method tests with spiked CuS (see section 2.2.4).
Figure 2.2. Reduced inorganic S during the reduction-reoxidation cycle. a) Chromium reducible sulfur (CRS) at selected time points compared to the background value of the oxic soil (CRS_{bkg}, indicated by shaded area). Error bars indicate the range of two independent digestions (for oxic soil: standard deviation of four independent digestions). b) Correlation between additionally formed CRS (CRS_{add} = CRS – CRS_{bkg}; error calculated by Taylor error propagation) and potential sulfide formation in the metal-spiked series. Potential sulfide formation was calculated from the difference between the total amount of extractable sulfate (MS and HS) or the maximum concentration of sulfate in solution (LS) and the concentration at day 10 or day 40 of soil reduction. Data points 1-6 in b) correspond to the numbered samples in a).

Assuming that CRS_{add} in the soil samples mainly originated from Cu sulfide phases, CRS_{add} values for all samples of the reduction-reoxidation cycle were therefore corrected using the recovery of CuS (CRS_{max} = CRS_{add} / 0.76). The resulting CRS_{max} values were considered to represent the maximum amount of newly formed sulfide present as Cu sulfide. The sum of CRS_{bkg} and CRS_{max} then represents the total amount of reduced inorganic S (CRS_{tot} = CRS_{bkg} + CRS_{max}).

2.3.3. Dynamics of Cu speciation in the solid phase

Sequential extraction of copper

The extractability of Cu in selected soil samples from the incubation series with the lowest (LS) and the highest (HS) Cu-to-sulfate ratio was characterized by a five-step sequential extraction (Table 2.3). The extracted fractions were reported as percentage of the total amount of Cu determined by XRF (Figure 2.3; values in Appendix A Table A.3). In the intermediary MS-series, only the first two extraction steps were conducted to confirm the observed trends.
Prior to and in the early stage of reduction, the fractionation of Cu was almost identical for all series. After the 2-day equilibration period, the major part of Cu was found in factions F2 ("easily mobilizable", \(\approx 50\%\)) and F3 ("organically bound", \(\approx 35\%\)). Within the first 10 days of reduction, Cu in F2 decreased to 15–21%, accompanied by a slight increase of Cu in F1 ("mobile") and F3. The remaining Cu was mainly found in F5 ("oxidizable") and the residual fraction (total Cu minus the sum of all extracted fractions). The extractability of Cu in the different series changed drastically with ongoing soil reduction. In the HS series, no Cu was extractable in F1–F3 after 40 days of reduction and Cu was almost completely repartitioned into fraction F5 (78%). In the MS and LS series, Cu was also depleted from F1 and F2 during soil reduction. However, the five-step extraction of the LS series revealed that 19% of the total Cu remained in fraction F3 even after 40 days of anoxic incubation - in contrast to the HS series. In parallel, an increase of Cu in the fraction F4 ("reducible") up to 15% was observed. During reoxidation a significant amount Cu in the HS series remained in fraction F5. In contrast, the initial Cu extractability was nearly reestablished in the LS series by the end of the reoxidation phase.
Cu K-edge X-ray absorption spectroscopy

The speciation of Cu in the soil matrix was characterized by X-ray absorption spectroscopy. Figure 2.4a and b show the normalized absorption spectra and the first derivative of the XANES region, which exhibits characteristic pre-edge and edge features depending on Cu redox state (Kau et al., 1987). In particular, the absorption edges of Cu(II) compounds (e.g., Cu(II)-clay) are located at significantly higher energies than the absorption edges of Cu(I) compounds (e.g., Cu$_{x}$S$_{prim}$) or Cu(0), as indicated by the maxima in the first derivative functions (Figure 2.4b). The shifts in the edge positions during soil reduction therefore suggested a transition of Cu(II) to Cu(I)/Cu(0) already within the early stages of the reduction phase and a reoxidation to Cu(II) upon aeration.

Quantitative information on Cu speciation was obtained by linear combination fitting (LCF) of the soil EXAFS spectra. In complex samples with several Cu species, different O-coordinated Cu(II) species such as Cu(II) adsorbed on the surfaces of clay minerals, metal oxides, and soil organic matter are difficult to distinguish by LCF analysis due to the structural similarity in their first-shell coordination (six-fold coordination by O in a distorted tetragonal configuration (Manceau and Matynia, 2010); see also Appendix A.1), weak second-shell contributions, and contributions from other Cu species. To limit the number of reference compounds for LCF, we therefore chose Cu(II)-clay as a proxy for O-coordinated adsorbed or complexed Cu(II). In analogy, Cu$_{x}$S$_{prim}$ was used as a proxy for S-coordinated Cu(I), including Cu(I) in metal sulfides and Cu(I) complexed by reduced organic S groups. Using metallic Cu as third reference spectrum, all sample spectra could be satisfactorily described by linear combination fitting. The spectra and corresponding LCF results are presented in Figure 2.4c-e and Table 2.4. The EXAFS spectrum of the 2-day equilibrated soil from the MS-series (MS-2dEQ) was predominantly fitted by Cu(II)-clay. The same Cu speciation can be assumed for the other freshly spiked and equilibrated soils (LS, HS). Because of the high affinity of Cu for natural organic matter (McBride et al., 1997), Cu(II) is expected to be predominantly bound by SOM. However, considering the high clay content of the soil (Table 2.1), clay minerals were probably also important sorbents for Cu(II) (Strawn et al., 2004). As already suggested by the XANES spectra, a major part of Cu was reduced to Cu(I) and Cu(0) already during the first 10 days of soil reduction. Metallic Cu
Figure 2.4. Cu K-edge X-ray absorption spectra of selected samples and reference compounds. a) Normalized XANES and b) first derivative of normalized XANES spectra (rebinned and smoothed in 3 cycles); c) k^3-weighted sample EXAFS spectra (solid lines) and corresponding spectra of the LCF (red symbols); d) Magnitude and e) real part of the Fourier transformed sample and LCF spectra (k-range=2.5–10 Å^{-1}, Kaiser Bessel window with dk=2.5 Å^{-1})
Table 2.4. LCF analysis of $k^3$-weighted EXAFS spectra (Figure 2.4c) over $k$-range 2.5–10 Å$^{-1}$. Individual fractions were constrained to the range 0–1, the sum of all fractions was not constrained.

<table>
<thead>
<tr>
<th>Incubation series</th>
<th>Cu(I)-clay (%)</th>
<th>Cu metal (%)</th>
<th>Cu$<em>x$S$</em>{prim}$ (%)</th>
<th>Sum (%)</th>
<th>NSSR $^a$ (%)</th>
<th>$\Delta$ Sulfide (mmol/kg)$^b$ x = 1</th>
<th>$\Delta$ Sulfide (mmol/kg)$^b$ x = 1.39</th>
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</thead>
<tbody>
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<td>low sulfate</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td>34</td>
<td>13</td>
<td>23</td>
<td>70</td>
<td>13</td>
<td>-0.7 ±0.2</td>
<td>-0.4 ±0.2</td>
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<tr>
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<td>35</td>
<td>53</td>
<td>102</td>
<td>2.2</td>
<td>-1.8 ±0.2</td>
<td>-1.2 ±0.2</td>
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<tr>
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<td>85</td>
<td>24</td>
<td>-0.7 ±0.3</td>
<td>-0.4 ±0.2</td>
</tr>
<tr>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2d EQ</td>
<td>72</td>
<td>1.5</td>
<td>8.8</td>
<td>82</td>
<td>14</td>
<td>+0.1 ±0.2</td>
<td>+0.2 ±0.2</td>
</tr>
<tr>
<td>10d RED</td>
<td>28</td>
<td>17</td>
<td>37</td>
<td>82</td>
<td>6.5</td>
<td>-1.3 ±0.3</td>
<td>-0.9 ±0.2</td>
</tr>
<tr>
<td>40d RED</td>
<td>12</td>
<td>--</td>
<td>97</td>
<td>110</td>
<td>18</td>
<td>-1.5 ±0.3</td>
<td>-0.3 ±0.3</td>
</tr>
<tr>
<td>14d REOX</td>
<td>36</td>
<td>1.2</td>
<td>60</td>
<td>98</td>
<td>19</td>
<td>-1.1 ±0.4</td>
<td>-0.3 ±0.3</td>
</tr>
<tr>
<td>high sulfate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10d RED</td>
<td>32</td>
<td>7.3</td>
<td>39</td>
<td>79</td>
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<td>-0.9 ±0.4</td>
<td>-0.4 ±0.3</td>
</tr>
<tr>
<td>40d RED</td>
<td>6.0</td>
<td>1.0</td>
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<td>107</td>
<td>16</td>
<td>+1.7 ±0.6</td>
<td>+2.9 ±0.6</td>
</tr>
</tbody>
</table>

$^a$ Normalized sum of squared residuals = $\sum_i (k^3 \chi_{exp} - k^3 \chi_{fit})^2 / \sum_i (k^3 \chi_{exp})^2$.

$^b$ Difference between the maximal amount of formed sulfide (CRS$_{max}$) and the amount of sulfide required to account for the Cu$_x$S fraction derived by LCF (assuming Cu$_x$S stoichiometry (x) of 1 (covellite) or 1.39 (spionkopite)) with uncertainties from Gaussian error propagation. Negative values indicate that the amount of sulfide derived from LCF exceeds the CRS$_{max}$ amount in the sample.

accounted for 13, 17 and 7% of the Cu in the LS, MS and HS series at day 10, and Cu$_x$S$_{prim}$ was fitted with 23, 37 and 39%, respectively. In the LS-series, the fraction of Cu(0) increased to 35% by the end of the reduction phase (day 40). In contrast, in the MS and HS series, Cu was almost completely transformed to primitive Cu sulfide after 40 days of reduction. The differences in Cu speciation after 40 days of soil reduction affected Cu reoxidation upon soil aeration. Whereas metallic Cu was fully oxidized after 14 days of aeration, a significant part of Cu sulfide was stable in contact with O$_2$. After 14 days of reoxidation, 28% of total Cu in the LS and 60% in the MS series were still fitted by primitive Cu sulfide.

The sum of the LCF fractions was typically less than 100% and lowest for the 10-days reduced soil samples (69-75%; Table 2.4). Considering that the fits were relatively good, the discrepancy in the fit sum was most probably due to lower first-shell EXAFS amplitudes of Cu species in the respective samples compared to the reference spectra used for LCF. Lower first shell amplitudes may be due to (i) higher first-shell disorder (of adsorbed/complexed or crystalline/amorphous Cu species), (ii) formation of Cu-NOM complexes with lower coordination numbers, (iii) partially
destructive interference arising from different first-shell atoms (e.g. O/N versus S in different species or in single species with different ligand atoms; see Appendix A Figure A.1b), or (iv) presence of very small (cluster-sized) Cu$_x$S or Cu(0) nanoparticles (Jayanetti et al., 2001). Furthermore, the high intensity of the EXAFS oscillations of metallic Cu, which represented a significant Cu fraction in the 10d RED samples, may have limited LCF sensitivity for other Cu species.

Comparison with additional XAS measurements collected at room temperature suggested that the LCF-derived fractions of Cu metal > 5% were highly reliable (Appendix A Figure A.8c). In contrast, discrepancies were observed with respect to Cu partitioning between the Cu(II)-clay and Cu$_x$S$_{prim}$ fractions, which may have resulted from beam-induced Cu reduction that could lead to an increase in Cu(I)-S species (Strawn and Baker, 2009; Manceau and Matynia, 2010). Therefore, the effective Cu$_x$S$_{prim}$ fractions may be at most 17% (absolute) lower than the values reported in Table 2.4 (for details see Appendix A.5). This interpretation was supported by the very good agreement between LCF-derived reduced Cu fractions (Cu$_x$S$_{prim}$ and Cu(0)) reported in Table 2.4 and the more recalcitrant Cu fractions (F4, F5 and RES) from the sequential extraction (see next section).

**Comparison of sequential extraction and EXAFS results**

In Figure 2.5, the sequentially extracted fractions of Cu (Figure 2.3) are compared to the Cu fractions from LCF analysis of EXAFS spectra (Table 2.4). The Cu(II)-clay fraction, which represented Cu(II) adsorbed to clay minerals, metal oxides, and soil organic matter, was expected to be extracted predominantly in the first three steps (F1–F3). Adsorbed Cu(II) occluded in aggregates of Fe- and Mn-oxides, which would also contribute to the Cu(II)-clay LCF fraction, was expected in fraction F4. However, the amount of Cu extracted in F4 after the 2-day equilibration phase was negligible, probably because the soil was artificially spiked with Cu. Therefore, the clear increase of the fraction F4 in the LS and HS series with soil reduction (Figure 2.3) was likely related to increasing fractions of Cu(0) (especially in the LS series) or Cu sulfide that became oxidized during extraction (extraction steps F4 and F5 were conducted
under oxic conditions). Otherwise, Cu sulfides are expected to be recovered mainly in the oxidizable fraction (F5). However, the efficiency of H$_2$O$_2$ to extract Cu sulfides can be reduced by thermal decomposition of H$_2$O$_2$ (Schultz et al., 1999) and strong reaction with soil organic matter. Thus, the sum of Cu sulfide and Cu(0) from LCF was compared to the sum of F4, F5, and the residual Cu amount (Figure 2.5c), although the residual fraction may also comprise minor amounts of Cu(II) contained in primary and secondary soil minerals.

The changes in Cu speciation during soil reduction as determined by EXAFS LCF analysis were in good agreement with changes in Cu fractionation in the sequential extraction. In general, the transformation of adsorbed Cu(II) into Cu sulfide and metallic Cu was accompanied by a decrease of the labile Cu fractions (F1, F2, F3) and an increase of Cu in the more recalcitrant fractions (F4, F5 and RES), as reflected by the correlations between the sums of respective fractions from XAS and from sequential extraction (Figure 2.5). In particular, the sum of the LCF-derived reduced Cu species, i.e., metallic Cu and Cu$_x$S very closely matched the sum of the extracted fractions F4 and F5, and the residual Cu (Figure 2.5c). The correlation between LCF-derived Cu(II) and the sum of the first three extraction steps exhibited some deviations from the 1:1 line that were mainly caused by samples after 10 days of reduction and were probably related to the fit uncertainties described in the previous section.
2.4 Discussion

In the following sections, we discuss the processes that control solid phase Cu speciation and dissolved Cu concentrations during different stages of the reduction-reoxidation cycle: (i) early stage of soil reduction prior to major sulfate reduction, (ii) period of major sulfate reduction, and (iii) soil reoxidation. Related to these three stages, we discuss the shift in solid phase Cu speciation between adsorbed Cu(II) and organically complexed Cu(I), metallic Cu, and Cu-sulfide, its impact on Cu solubility, and general implications for Cu dynamics in periodically flooded soils.

2.4.1. Cu dynamics in the solid and solution phase during soil reduction

Solid phase speciation of Cu during early stages of soil reduction

X-ray absorption spectroscopy results clearly showed a fast reduction of soil Cu(II) within the first 10 days of anoxic incubation. Sequential extractions suggested that predominantly Cu(II) from the mobile and easily mobilizable fractions (F1+F2) repartitioned into the more recalcitrant Cu fractions (F4, F5, residual). The increase of Cu in fraction F5 (“oxidizable”) together with the LCF results of 23–39% Cu$_{S_{\text{prim}}}$ suggested that Cu reduction was partially linked to Cu sulfide formation (Pattrick et al., 1997; Luther et al., 2002). However, the maximum amounts of sulfide (CRS$_{\text{max}}$) formed by that time were too low to balance the whole amount of Cu present as S-coordinated Cu(I) according to LCF analysis of Cu EXAFS spectra. Luther et al. (2002) showed that reaction of Cu(II) with equimolar amounts of sulfide led to Cu reduction and formation of aqueous Cu-(poly)sulfide complexes and polynuclear clusters as intermediates during Cu sulfide precipitation. These aqueous clusters and freshly formed primitive Cu sulfide phases are thought to have a Cu/S ratio close to unity (Pattrick et al., 1997; Rozan et al., 2000; Luther et al., 2002). Based on this ratio, the fitted fractions of Cu$_{S_{\text{prim}}}$ correspond to 1.0–1.7 mmol kg$^{-1}$ sulfide, exceeding the experimentally derived CRS$_{\text{max}}$ by 0.7–1.3 mmol kg$^{-1}$ (Table 2.4). Even if we assumed a higher Cu/S ratio of 1.39 (corresponding to spionkopite (Cu$_{39}$S$_{28}$)) as an upper limit for the Cu/S ratio in amorphous Cu$_{S}$ (Shea and Helz, 1989; Pattrick et al., 1997), or take into account that effective Cu$_{S_{\text{prim}}}$ fractions could be up to 17% (absolute)
lower than reported in Table 2.4 (see Appendix A.5), the LCF-derived fractions of S-coordinated Cu(I) still exceeded maximum sulfide formation after 10 days of reduction at least in the MS-series (and especially after 40 days in the LS-series; see next section) (Table 2.4 and Appendix A Table A.4). Furthermore, the discrepancy in the mass balance between S-coordinated Cu and formed sulfide may be even higher, since possible metal-sulfide formation by other chalcophile trace metals like Cd and Pb or by Fe were not taken into account. Although, the solubility products of these metal sulfides are much higher than that of CuS (Morse and Luther, 1999), kinetic constraints limiting Cu supply may cause minor fractions of these metals to react with sulfide (Weber et al., 2009a; Hofacker et al., 2013). In particular, less than 0.5% of the reducible soil Fe (∼170 mmol kg\(^{-1}\) poorly crystalline Fe(III) (oxyhydr)oxides, corresponding to ∼20% of total soil Fe (Dittmar et al., 2007)) would already suffice to balance \(\text{CRS}_{\text{max}}\) after 10 days of reduction. Therefore, processes other than precipitation of Cu-sulfide must have contributed to the high amounts of S-coordinated Cu(I) in the early stage of soil reduction.

Fast reductive transformation of Cu(II) to Cu(I) may have proceeded by either microbial reduction (Wakatsuki, 1995) or by abiotic processes like Cu(II) reduction by dissolved Fe\(^{2+}\) (Matocha et al., 2005) or natural organic matter (Pham et al., 2012). In the absence of significant amounts of biogenic sulfide, complexation by reduced organic S groups of bacterial cell surfaces, extracellular polymeric substances (EPS) or NOM may have stabilized Cu(I) against disproportionation. Many studies on estuarine systems showed that thiol-containing compounds (e.g., glutathione) play a key role in the stabilization of Cu(I) (Boulegue et al., 1982; Leal and van den Berg, 1998). Also complexation of other chalcophile trace metals like Cd(II) and Hg(II) by thiol-groups of SOM and DOM has been shown previously (Karlsson et al., 2005; Skyllberg et al., 2006), and it is therefore reasonable to assume that the very soft Lewis acid Cu(I) has a similar affinity for reduced organic S groups (\(S_{\text{org}}\)) of NOM (Smith et al., 2002). Additionally, Cu(I) may be bound by cysteine (R-SH) and methionine (R-S-R) residues in bacterial Cu transport and resistance proteins (Rubino and Franz, 2012). Weber et al. (2009b) found some indication for Cu(I) complexation by reduced S groups of bacterial cuproproteins within very short time (1 day) after soil flooding and onset of reduction. Potential formation of Cu(I)-\(S_{\text{org}}\) complexes in wetland soils was recently also postulated by Lin et al. (2010) and Hofacker et al. (2013).
To examine whether the observed discrepancy between LCF-derived S-coordinated Cu(I) and maximal sulfide formation can be explained by complexation of Cu(I) with reduced organic S groups, LCF analysis was repeated, including different Cu(I)-S$_{org}$ references with various first shell coordination (Appendix A Table A.1). The fits were conducted with each Cu(I)-S$_{org}$ reference individually, and the fraction of Cu$_x$S$_{prim}$ was fixed based on the corresponding CRS$_{max}$ assuming a Cu:S stoichiometry of 1:1. For each sample, the fitted fractions obtained from the four LCF fits (Appendix A Table A.5 and Figure A.9) were averaged and the results are given in Table 2.5. The sum of the fractions of Cu(I)-S$_{org}$ and Cu$_x$S$_{prim}$ in these fits closely matched the Cu$_x$S$_{prim}$ fraction of fits without organic Cu-S references (Tables 2.4 and 2.5), while the Cu(II)-clay and Cu(0) fractions remained unchanged (differences < 3%; absolute) and the quality of the fits was comparable (increase in NSSR < 1%; absolute). These results indicated that complexation of Cu(I) by reduced organic S groups contributed to the total fraction of S-coordinated Cu(I). The results also showed that unambiguous distinction between amorphous Cu-sulfide and Cu(I)-S$_{org}$ by EXAFS spectroscopy in complex soil samples can be difficult. This can be attributed to the similar local Cu-S coordination in amorphous Cu-sulfide and Cu(I)-S$_{org}$ complexes (Appendix A Table A.1) and hence similar EXAFS features (Appendix A Figure A.2), and to the presence of other interfering Cu species, in particular metallic Cu with high EXAFS amplitudes. EXAFS analysis revealed the formation of 7.5–17% Cu(0) within the first 10 days of soil reduction. Metallic Cu may form by the reaction of Cu(II) with dissolved Fe$^{2+}$ (Matocha et al., 2005) or adsorbed Fe$^{2+}$ (Genovese and Mellini, 2007), or Fe(II)-bearing mineral phases like green rust or ferrous clay (Ilton et al., 1992; O’Loughlin et al., 2003). Recently, metallic Cu formation prior to sulfate reduction has been observed in microcosm experiments with a contaminated river floodplain soil (Weber et al., 2009a; Hofacker et al., 2013). In these studies, Cu(0) formation has been attributed to a bacterial Cu detoxification process leading to the export of Cu(I) followed by its disproportionation to Cu(0) and Cu(II) in the absence of Cu(I)-stabilizing ligands.
Table 2.5. Average from LCF analysis of $k^3$-weighted EXAFS spectra ($k$-range 2.5–10 Å$^{-1}$) using different references for Cu(I)-Sorg (Cu(I)-Carbamate, Cu(I)-GSH, Cu(I)-S-trigonal, Cu(I)-CopC) (mean ± standard deviation; n=4). The fraction of Cu$S_{prim}$ was fixed based on the maximal sulfide formation (CRS$_{max}$) assuming a Cu to S ratio of 1:1.

<table>
<thead>
<tr>
<th>Incubation series</th>
<th>Cu(II)-clay (%)</th>
<th>Cu metal (%)</th>
<th>Cu$S_{prim}$ (%)</th>
<th>Cu(I)-Sorg a (%)</th>
<th>Sum a (%)</th>
<th>NSSR ab (%)</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
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</tr>
<tr>
<td>10d RED</td>
<td>32 ±1.2</td>
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<td>8.0</td>
<td>16 ±5.3</td>
<td>69 ±4.2</td>
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<td>30 ±8.4</td>
<td>89 ±6.8</td>
<td>2.7 ±0.4</td>
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<tr>
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<td>11</td>
<td>17 ±5.1</td>
<td>84 ±3.6</td>
<td>23 ±1.3</td>
</tr>
<tr>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2d EQ</td>
<td>72 ±0.0</td>
<td>1.4 ±0.0</td>
<td>10</td>
<td>0.1 ±0.2</td>
<td>83 ±0.2</td>
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</tr>
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<tr>
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<td>19 ±1.8</td>
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<tr>
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<td>93 ±6.5</td>
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</tr>
<tr>
<td>10d RED</td>
<td>31 ±0.8</td>
<td>7.5 ±0.5</td>
<td>18</td>
<td>15 ±2.5</td>
<td>72 ±2.1</td>
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<td>13 ±3.7</td>
<td>117 ±2.8</td>
<td>15 ±0.1</td>
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</table>

* Single values are reported in Appendix A Table A.5. 
** Normalized sum squared residuals = $\sum (k^3\chi_{exp} - k^3\chi_{fit})^2 / \sum (k^3\chi_{exp})^2$.

Solid phase speciation of Cu during major sulfate reduction

With ongoing soil reduction, Cu(0) increased up to 35% in the LS-series (Table 2.4), due to the low biogenic sulfide availability. In contrast to the LS-series, no Cu(0) was found after 40 days of reduction in the MS and HS series. At this time sulfate reduction was completed and Cu was found almost entirely in the S-bound fraction, confirming that metallic Cu is sulfidized during sulfate reduction and biogenic sulfide release (Weber et al., 2009a; Hofacker et al., 2013).

In addition to the sulfidization of metallic Cu, sulfate reduction in the MS and HS series also resulted in transformation of remaining Cu(II) to Cu-sulfide. In the corresponding Fourier-transformed EXAFS spectra, no peak was observed at 3–4 Å (Figure 2.4d and e) where evolved Cu$_3$S and covellite exhibit a marked second-shell peak from Cu-Cu backscattering (Patrick et al., 1997). This suggested that the Cu-sulfide formed during soil reduction remained amorphous, which may be attributed to inorganic and organic ligands that hinder the coagulation and crystal growth of Cu-sulfide phases (Horzempa and Helz, 1979; Shea and Helz, 1987). Only in the 40d RED sample of the HS series, a minor contribution of a second shell was visible. Interestingly,
this second-shell peak more closely resembled the frequency and phase shift of the second-shell peak of chalcopyrite (CuFeS$_2$) than covellite (Appendix A Figure A.10), suggesting that Cu may have become partially incorporated into mixed Cu-Fe sulfide precipitates in the presence of excess sulfide. Parkman et al. (1999) observed that the reaction of freshly-precipitated mackinawite (FeS) with dissolved Cu(II) led to the formation of a chalcopyrite-type phase within 24 h. This process may have been especially relevant in the HS series, where the rate of sulfate reduction (and thus sulfide release) was twice as high as in the LS and MS series (see Appendix A Figure A.5a). Considering the higher amount of available sulfide in the HS series and the large pool of reducible Fe (about 170 mmol kg$^{-1}$), precipitation of mackinawite may have temporarily dominated over formation of thermodynamically more stable Cu$_x$S phases (Shea and Helz, 1989) due to slow release of adsorbed Cu(II) (Shi et al., 2005).

As discussed for the 10d RED samples, the fractions of Cu$_x$S in the LS and MS series after 40 days of reduction significantly exceeded the maximal available amounts of sulfide by 1.5–1.8 mmol kg$^{-1}$ (Table 2.4). Fixing the Cu$_{S\text{prim}}$ fractions to the values estimated from the CRS$_{\text{max}}$, 30 and 26 % of the total Cu (1.1 and 1.3 mmol kg$^{-1}$) could be attributed to Cu(I)-S$_{\text{org}}$ (Table 2.5). These amounts can be used as a rough estimate of the capacity of reduced organic S groups for Cu(I) stabilization (assuming a 1:1 stoichiometry for Cu:S$_{\text{org}}$). For an organic peat soil, Skyllberg et al. (2006) concluded from S and Hg XAS that about 60% of the organic S was reduced S, and that 20–30% of the reduced organic S corresponded to high-affinity sites for soft metal cations. Using these fractions, the amount of high-affinity organic S groups in our soil was estimated to range between 1.1 and 1.7 mmol kg$^{-1}$ (organic S calculated by subtracting CRS and sulfate from total soil S), which is in very close agreement with the amount of S$_{\text{org}}$ estimated from our Cu XAS data (1.1 and 1.3 mmol kg$^{-1}$).

**Effects of solid phase Cu speciation on dissolved Cu during soil reduction**

The trends in dissolved Cu concentrations (Figure 2.1f) reflected the changes in Cu solid phase speciation. After the initial sharp increase in Cu concentration due to the addition of lactate, Cu decreased rapidly with the beginning of the reduction period. Considering the high thermodynamic stability of Cu sulfides and the micromolar levels of dissolved Cu at the onset of
soil reduction, the rapid decrease of dissolved Cu was most probably due to reaction with minute amounts of sulfide released during initial slow sulfate reduction, which is in line with the EXAFS results and the concomitant increase of the oxidizable Cu fraction (Figure 2.3, fraction F5). In addition, the EXAFS results also pointed to a fast biotic or abiotic reduction of dissolved Cu\(^{2+}\) to the more chalcophile Cu\(^{+}\) followed by complexation by reduced organic S groups, as well as to the precipitation of metallic Cu, which may have also contributed to the rapid depletion of dissolved Cu. These processes can be considered to control Cu solution dynamics during reduction and interfering with the possible release of the mobile and easily mobilizable Cu fraction (50% of the total Cu, Figure 2.3) by competitive readsorption of dissolved Fe\(^{2+}\) and Mn\(^{2+}\), as it is known for other trace metals like Co, Ni, and Cd (Zachara et al., 2001; Weber et al., 2009a), or by the continuous release of DOM (Grybos et al., 2007). The importance of Cu(I)-S\(_{org}\) complexes and Cu(0) formation for Cu solution dynamics became especially evident in the LS series during ongoing reduction. In contrast to the MS and HS series, where Cu(0) sulfidization and further Cu(II) sequestration into Cu\(_{x}\)S kept Cu solution concentrations low, the amount of biogenic sulfide in the LS series was too small to effectively stabilize Cu by Cu\(_{x}\)S precipitation. Nevertheless, dissolved Cu concentrations remained low until the end of the reduction period in this series. The effect of sulfate availability on dissolved Cu was only apparent by the time point where Cu concentrations fell below the detection limit (0.05 \(\mu\)M), which was after day 40, day 30, and day 15 in the LS, MS, and HS series, respectively.

2.4.2. **Cu dynamics in the solid and solution phase during soil reoxidation**

**Solid phase speciation of Cu during soil reoxidation**

Exposing the reduced soils to O\(_2\) led to changes in solid phase Cu speciation and extractability depending on the predominant Cu species present after soil reduction. Metallic Cu(0), which was still present in the LS series due to the lack of sulfidization, was fully oxidized within 14 days. This is in agreement with a laboratory study by Kanninen et al. (2008), who showed that Cu(0) nanoparticles were rapidly oxidized in aerated solutions within minutes. In contrast, more than half of the S-coordinated Cu in the LS and MS series persisted over 14 days of soil reoxidation (Table 2.4). Considering the CRS\(_{max}\) amounts after 14 days of reoxidation, Cu(I)-S\(_{org}\) may have
contributed to about 20% of the remaining fraction of S-coordinated Cu(I) (Table 2.5). This suggests that Cu(I)-S$_{org}$ complexes are only slowly oxidized upon exposure to O$_2$, as has previously been inferred from the presence of Cu(I)-thiol complexes in oxic estuarine waters (Leal and van den Berg, 1998; Laglera and van den Berg, 2003).

After 28 days of soil reoxidation, CRS$_{tot}$ was still higher than the initial background concentration. The fractions of Cu extracted in the oxidizing step (F5) of the sequential extraction (0.4 mmol kg$^{-1}$ in LS; 1.9 mmol kg$^{-1}$ in HS) closely matched the corresponding amounts of CRS$_{max}$, suggesting that Cu extracted in this step can be attributed to persisting Cu$_{x}$S with a 1:1 stoichiometry. This further implies that other metal sulfides precipitated in the HS series with excess sulfide, such as mackinawite (FeS), were readily reoxidized upon aeration, while Cu sulfide was more resistant. This observation is in line with results from studies with pure metal sulfides in aerated water, which showed that FeS oxidizes much more rapidly than Cu sulfides (Simpson et al., 1998; Sukola et al., 2005). A high stability of Cu sulfides in the presence of O$_2$ has also been inferred from the presence of aqueous Cu sulfide clusters in oxic river waters (Rozan et al., 2000).

Effects of solid phase Cu speciation on dissolved Cu during soil reoxidation

The dynamics of dissolved Cu during soil reoxidation (Figure 2.1f) could be attributed to changes in solid-phase Cu speciation. Dissolved Cu concentrations significantly increased in all series due to the oxidative dissolution of reduced Cu species, concomitant to the decrease in the LCF-derived S-coordinated Cu and metallic Cu fractions, as well as the shift of Cu fractionation from the more recalcitrant to the more readily extractable fractions. In the LS series dissolved Cu initially increased to a slightly higher level than in the other series. Considering that the 40d RED sample from the LS series contained the highest fraction of metallic Cu, this may indicate that Cu(0) dissolved more rapidly than the S-coordinated Cu(I) species that dominated Cu speciation in the MS and HS series after soil reduction.

Although all Cu(0) and about half of the Cu-sulfide was oxidized within 4 weeks of reoxidation, only 0.1–0.2% of total Cu was released into solution during this period, suggesting that Cu(II) adsorption and precipitation processes effectively counteracted the oxidative dissolution of
reduced Cu species. This was also observed in studies on metal dynamics during the oxidation of sulfidic sediment suspensions (Calmano et al., 1994; De Jonge et al., 2012). Nevertheless, dissolved Cu concentration after soil reoxidation exceeded the concentrations after soil equilibration by up to factor 2- to 5 and despite their decrease after 2 weeks still remained high even after 4 weeks of reoxidation. This was possibly due to the 2- to 3 times higher DOC concentration compared to the initial values after equilibration.

2.4.3. Implications for Cu dynamics in periodically flooded soils

In the present study, we conducted batch experiments in which suspended paddy soil, adjusted to different sulfate levels, was equilibrated for two days with added Cu(II) before undergoing a soil reduction-reoxidation cycle. The general trends during soil reduction and concomitant solid-phase Cu speciation changes were in excellent agreement with earlier work conducted with a riparian floodplain soil (Weber et al., 2009a; Hofacker et al., 2013). This soil had been contaminated with Cu from metal mining for centuries, and soil reduction experiments were conducted in soil microcosms under a realistic flooding regime. We therefore conclude that the findings from the present study provide reliable insight into processes relevant for Cu dynamics in periodically flooded wetland soils.

The amount of biogenic sulfide formed during soil reduction is a key factor with respect to changes in the solid phase speciation and solubility of Cu during soil reduction and subsequent reoxidation. Our results emphasize the key role of the amount of available sulfate which constrains the maximum amount of sulfide formed by microbial sulfate reduction during soil flooding, since mineralization of organic S under anoxic conditions is typically too slow to provide a significant alternative source of biogenic sulfide over a single flooding event. However, also in the case of relatively high sulfate availability, biogenic sulfide formation during soil reduction may be limited if the flooding period is not long enough to reach major sulfate reduction. Dominant factors that limit the rate of microbial sulfate reduction include lower temperature (Bak and Pfennig, 1991; Hofacker et al., 2013), limited availability or low biodegradability of respirable organic C (Jakobsen and Postma, 1994; Harris et al., 2006), competitive inhibition of sulfate reduction by Fe(III) reducing bacteria (Lovley and Phillips,
slow transport of solutes to particle-bound sulfate-reducing bacteria (Pallud and Van Cappellen, 2006), or localized sulfate-reduction in microniche (Widerlund and Davison, 2007). Our results revealed that if sulfide formation is limited either by low sulfate availability or due to an inhibition of sulfate reduction, soil redox state may nevertheless be low enough to allow for the reduction of Cu(II) to Cu(I) by biotic or abiotic processes. The formed Cu(I) may be stabilized against disproportionation through complexation by reduced S groups of natural organic matter. Depending on the solubility of the reduced S-containing organic substances, the formation of Cu(I)-S$_{\text{org}}$ complexes may either enhance or decrease Cu solubility in soils. Our study further confirmed that in the absence of sufficient amounts of sulfide or other stabilizing ligands, metallic Cu(0) formed by Cu(I) reduction or disproportionation can be an important Cu species. On the other hand, under conditions of high sulfate availability and sufficient sulfate reduction during soil flooding, Cu-sulfide precipitation largely controls Cu solid phase speciation and limits Cu solubility in flooded soil.

Although Cu reduction and Cu-sulfide formation is in general expected to decrease the solubility of Cu, previous studies reported that the formation of mobile Cu(0) and Cu sulfide colloids may also drive Cu mobilization upon soil flooding (Weber et al., 2009b; Hofacker et al., 2013). Conversely, our results and sediment resuspension studies (Calmano et al., 1994; Du Laing et al., 2007) indicate that elevated Cu concentrations in the pore-water can also be expected during soil reoxidation, i.e., due to pore-water drainage and aeration or intrusion of oxic water. Cu concentrations rapidly increased during reoxidation due to the oxidative dissolution of reduced Cu species, and remained high even over a period of four weeks. Our study further suggested that this temporal Cu release is expected to be more pronounced if soil Cu speciation at the end of the reduction phase is dominated by metallic Cu, because oxidative dissolution of metallic Cu proceeds more rapidly than dissolution of Cu-sulfide. In combination, these results suggest that the release of Cu from soil into surface and groundwater in colloidal and dissolved form can be triggered by soil flooding and drainage, respectively.

This study demonstrated that metallic Cu and Cu(I) complexed by reduced organic S groups may be important Cu species in temporarily reduced soils, in addition to Cu-sulfide. Further research is needed with respect to Cu redox and sorption interactions with natural organic matter,
namely the extent to which Cu(I) can be stabilized by complexation by NOM. Due to its abundance relative to other chalcophile metals such as Hg, Ag, or Cd and the high stability of Cu-sulfide minerals compared to other transition metal sulfides, the speciation of Cu and its interaction with limited amounts of biogenic sulfide are expected to strongly influence the degree to which other trace metals form metal sulfides, as observed in microcosm experiments with a contaminated floodplain soil (Weber et al., 2009a; Hofacker et al., 2013). The interaction of Cu with biogenic sulfide and its effect on the transformation of other trace elements under sulfate-reducing conditions warrants further study.

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2.5. References


3. **Redox-controlled changes in cadmium solubility and solid-phase speciation in a paddy soil as affected by reducible sulfate and copper**

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**Abstract**

The solubility of Cd in contaminated paddy soils and hence the potential uptake of Cd by rice plants is an important food safety issue, as Cd is highly toxic to humans. Here, we investigated the solution and solid-phase dynamics of Cd in a paddy soil spiked with ~20 mg kg⁻¹ Cd during 40 days of soil reduction followed by 28 days of reoxidation as a function of the amounts of sulfate available for microbial reduction and of Cu that competes with Cd for precipitation with biogenic sulfide. At an excess of sulfate over (Cd+Cu), dissolved Cd decreased during microbial sulfate reduction and Cd was almost completely transformed into poorly soluble phases, which were identified as nanometer-sized and/or poorly crystalline Cd-sulfide using Cd K-edge X-ray absorption spectroscopy (XAS). The extent of Cd-sulfide precipitation and Cd immobilization decreased with decreasing reducible sulfate and increasing Cu contents, even if sulfate was present in excess over Cd. When both Cu and Cd exceeded the amount of reducible sulfate, dissolved Cd and the mobilizable Cd fraction remained elevated after 40 days of soil reduction. Nevertheless, XAS still indicated the formation of a minor fraction of Cd-sulfide under these conditions in addition to other S-coordinated Cd species, probably Cd bound to reduced organic S groups or to the surface of other metal-sulfide phases. During soil reoxidation, all
S-coordinated Cd species were readily transformed back into more soluble O-coordinated species. Overall, our data suggest that when the amounts of Cd and competing chalcophile cations such as Cu significantly exceed the amount reducible sulfate, Cd solubility and extractability may remain high even under anoxic conditions. Therefore, in multi-metal contaminated paddy soils with low amounts of reducible sulfate, Cd may remain labile during soil flooding, enhancing the risk for Cd transfer into rice.

3.1. Introduction

Cadmium (Cd) contamination of paddy soils due to the application of Cd-containing phosphate fertilizers or the use of contaminated irrigation water has been reported for several Asian countries, including China, Japan, Bangladesh, Thailand, Taiwan and Korea (Chen, 1991; Kashem and Singh, 1999; Simmons et al., 2005; Lee, 2006; Arao et al., 2009; Williams et al., 2009). Compared to other trace metals, Cd is rather mobile in soils (Alloway, 1995). Consequently, Cd is more readily taken up by rice plants, where it can be translocated into the grain and thereby enter the human food chain (Uraguchi et al., 2009; Meharg et al., 2013). Long-term consumption of Cd-contaminated rice can cause serious human health problems such as the itai-itai disease (Nordberg, 2004). Because rice is the staple food for about half of the world's population, Cd contamination of paddy soils and Cd uptake by rice has become an important food safety issue (Meharg et al., 2013).

Paddy soils are characterized by periodically changing redox conditions during rice cultivation, with prolonged flooding during the growing season and soil drainage prior to harvest (Koegel-Knabner et al., 2010). Changes in soil redox state affect the solid phase speciation and solubility of Cd and its availability for uptake by rice. During soil reduction, Cd may be mobilized by reductive dissolution of Mn(III/IV)- and Fe(III)-(oxyhydr)oxide sorbent phases and concomitant increases in dissolved Mn$^{2+}$ and Fe$^{2+}$ that compete with Cd for sorption sites (Zachara et al., 2001; Weber et al., 2009a). On the other hand, a decrease in Cd solubility or extractability during paddy soil reduction was observed in numerous laboratory studies, which was attributed to the precipitation of Cd-sulfide under sulfate reducing conditions (Cornu et al., 2007; de Livera et al., 2011; Huang et al., 2013). Also lower Cd transfer into rice plants and grains in flooded than
3.1 Introduction

drained soil was suggested to be due to Cd-sulfide formation under reducing conditions (Bingham et al., 1976; Arao et al., 2009).

Direct spectroscopic insight into solid-phase Cd speciation and the relevance of Cd-sulfide formation in flooded paddy soils is limited. Recently, Khaokaew et al. (2011) used Cd K-edge X-ray absorption spectroscopy (XAS) to monitor changes in Cd speciation during flooding of a highly contaminated alkaline paddy soil (142 mg kg\(^{-1}\) Cd). Over 150 days of flooding, Cd-carbonate represented the dominant Cd fraction, and only a minor fraction of the soil Cd became sequestered into Cd-sulfide. In a microcosm flooding experiment with a contaminated floodplain soil (30 mg kg\(^{-1}\) Cd) (Weber et al., 2009a; Weber et al., 2009b), on the other hand, we observed the formation of Cd-containing Cu-rich metal-sulfide nanoparticles in pore water by electron microscopy and concluded from soil extractions that most Cd became sequestered as Cd-sulfide during soil sulfate reduction. Our results, however, also suggested that the amount of sulfate available for microbial reduction may limit the extent to which individual trace metals are sequestered into poorly soluble metal-sulfides (Weber et al., 2009a).

Paddy soils often contain low amounts of reducible sulfate, with mean sulfate concentrations in different regions ranging between 0.06 to 6.6 mmol kg\(^{-1}\) (Lefroy et al., 1992). Although molar Cd concentrations in contaminated paddy soils will rarely exceed the amount of reducible sulfate, different chalcophile metals (e.g., Cu, Hg, Pb) may effectively compete with Cd for reaction with biogenic sulfide and thereby counteract Cd immobilization if reducible sulfate is low relative to the total chalcophile metal content (Weber et al., 2009a; Hofacker et al., 2013). Because the thermodynamic stability of CuS is several orders of magnitude higher than that of CdS (Millero, 1986) and because Cu typically occurs at much higher concentrations than Cd in soils, high concentrations of Cu relative to reducible sulfate may strongly affect the extent to which Cd can be sequestered into Cd-sulfide during soil flooding. The influence of Cu on Cd-sulfide formation in turn presumably depends on factors like metal desorption or precipitation kinetics as well as the stability of other metal phases (Weber et al., 2009a).

The extent of Cd-sulfide formation during paddy soil flooding may also be relevant with respect to Cd remobilization during paddy soil drainage and oxidation. For example, in experiments with a field-contaminated and a metal-spiked paddy soil it was observed that Cd release upon soil
reoxidation was lower when sulfate was added prior to anoxic incubation, which was related to a greater precipitation of Cd-sulfide during the reduction period (de Livera et al., 2011). A higher solubility of Cd during soil drainage is undesirable because it facilitates Cd transfer into the rice plant, increasing the risk of Cd accumulation in the grain (Arao et al., 2009; Yang et al., 2009; Hu et al., 2013).

The aim of the present study was to evaluate the effects of the contents of reducible sulfate and Cu in a non-calcareous paddy soil on changes in Cd solubility and solid-phase speciation. A incubation experiment was conducted with an uncontaminated paddy soil from Bangladesh that was spiked with ~20 mg kg\(^{-1}\) Cd and adjusted to different sulfate and Cu contents prior to incubation. Changes in dissolved Cd over the course of a 40-day reduction period and a subsequent 28-day reoxidation period were related to variations in Cd solid-phase speciation as determined by sequential extractions and Cd \(K\)-edge X-ray absorption spectroscopy (XAS). With respect to the potential effect of Cu on Cd-sulfide formation, the results were also interpreted in relation to concomitant changes in dissolved Cu concentration and solid-phase Cu speciation presented in a previous study (Fulda et al., 2013). Notably, very few studies to date used XAS to obtain direct insights into solid-phase Cd speciation in natural soils or sediments (Carroll et al., 2002; Khaokaew et al., 2011). Compared to these studies, the present work addresses Cd speciation in a paddy soil at a much lower Cd level (~20 mg kg\(^{-1}\)) that is not only relevant with respect to rice production on paddy soils (Jung and Thornton, 1997; Williams et al., 2009) but also with respect to contaminated riparian soils (Schulz-Zunkel and Krueger, 2009; Weber et al., 2009a).

### 3.2. Materials and Methods

#### 3.2.1. Soil material

A large batch of topsoil (~150 kg, 0–10 cm depth) of a non-calcareous Hydragric Anthrosol (Hypereutric, Siltic) was collected from a rice paddy field near Sreenagar (Munshiganj district, Bangladesh) (Dittmar et al., 2007). The soil material was oven-dried at 60 °C, broken up into soil aggregates < 2 cm (jaw crusher; Retsch, Germany), mixed, and characterized for basic physical and chemical properties. The soil had a silty clay loam texture, a weakly acidic pH of 6.2 (in
10 mM CaCl$_2$) and contained 2.3% organic carbon (Fulda et al., 2013). Total Cu (0.92 mmol kg$^{-1}$) and Cd (<0.01 mmol kg$^{-1}$) concentrations were in the range of background values reported for Bangladesh paddy soils (Ali et al., 2003). The amount of sulfate available for anaerobic microbial respiration was 2.09 mmol kg$^{-1}$ (NaHCO$_3$ extraction), which is within the range of mean concentrations reported for paddy soils from several countries (Lefroy et al., 1992). A more detailed description of the field site and of soil properties is given elsewhere (Dittmar et al., 2007; Fulda et al., 2013).

### 3.2.2. Incubation experiment

Four different incubation series were conducted in which the soil was adjusted to 0.2 mmol kg$^{-1}$ Cd and different Cu and sulfate concentrations as summarized in Table 3.1. For each series, 20-g aliquots of dry soil material were placed into 120 mL crimp vials (three replicates per sampling time) and suspended in 40 mL mixtures of CdCl$_2$, CuCl$_2$, CaSO$_4$, and CaCl$_2$ solution to adjust the levels of Cd, Cu, and sulfate and to maintain a constant background concentration of 5 mM Cl$^-$. In one treatment (low-sulfate series) the soil was washed three times for 1 h with 80 mL of 1 mM CaCl$_2$ solution to remove the exchangeable sulfate before addition of metal spike solution. During the soil washing procedure, 2.02 ± 0.03 mmol kg$^{-1}$ sulfate (mean ± standard deviation, n=9) were removed from the soil. Considering the total amount of exchangeable sulfate in the untreated soil (2.09 mmol kg$^{-1}$) (Fulda et al., 2013), the residual amount of sulfate available for anaerobic respiration in the low-sulfate series was 0.06 ± 0.03 mmol kg$^{-1}$ (Table 3.1). The fraction of major cations and trace elements removed during soil washing was negligible (<1% of the respective total element content).

After addition of the spike solutions, the incubation vials were equilibrated for 2 days on a horizontal shaker in contact with air ("equilibration phase", EQ). To stimulate microbial activity after the initial equilibration phase, a supplementary C source (5 mM Na-lactate) and 2.5 mL of unspiked soil suspension (20 g soil equilibrated for 2 days with 40 mL of 2.5 mM CaCl$_2$ background solution), containing the natural microbial community were added as inoculum. The vials were subsequently sealed with a butyl rubber septum and, after replacing the headspace
Table 3.1. Total Cd, Cu, and sulfate concentrations in the spiked soils. The untreated soil contained 0.92 mmol kg$^{-1}$ Cu and 2.09 mmol kg$^{-1}$ extractable sulfate.

<table>
<thead>
<tr>
<th>Incubation series</th>
<th>Abbreviation</th>
<th>Cu</th>
<th>Cd</th>
<th>Sulfate</th>
<th>(Cd+Cu)/sulfate ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>low sulfate - Cd and Cu spike</td>
<td>LS-HCu</td>
<td>4.42</td>
<td>0.17</td>
<td>0.06</td>
<td>77</td>
</tr>
<tr>
<td>medium sulfate - Cd and Cu spike</td>
<td>MS-HCu</td>
<td>4.43</td>
<td>0.18</td>
<td>2.09</td>
<td>2.2</td>
</tr>
<tr>
<td>high sulfate - Cd and Cu spike</td>
<td>HS-HCu</td>
<td>4.44</td>
<td>0.19</td>
<td>5.92</td>
<td>0.5</td>
</tr>
<tr>
<td>medium sulfate - only Cd spike</td>
<td>MS-LCu</td>
<td>0.92</td>
<td>0.18</td>
<td>2.09</td>
<td>0.5</td>
</tr>
</tbody>
</table>

a Total element concentration after incubation determined by XRF (mean ± standard deviation; n=11); b NaHCO$_3$ extractable sulfate minus sulfate extracted during soil washing (uncertainty from Gaussian error propagation).

With nitrogen gas, the suspensions were incubated for up to 40 days at 28.0 ± 1.2 °C with continuous end-over-end shaking (“reduction phase”, RED). Subsequently, the vials were opened and the suspensions were allowed to reoxidize for up to 4 weeks by horizontal shaking in contact with air (“reoxidation phase”, REOX).

### 3.2.3. Sampling and analysis

All sample processing was carried out in a glovebox (Braun, Germany) under N$_2$ atmosphere (O$_2$ < 1 ppm). For solution and solid-phase analyses, three incubation vials from each incubation series were taken after the EQ-phase, and at different time points within the RED-phase (day 1, 5, 10, 15, 20, 30, and 40) and the REOX-phase (day 2, 7, 14, and 28), respectively. The soil suspensions were transferred into centrifuge vials and centrifuged (10 min at 3566 g). The supernatants were decanted and passed through 0.2-μm nylon filters (Opti-Flow; Wicom, Germany). Solution pH and E$_h$ were measured in the unfiltered supernatants. Aliquots of the filtered supernatants were frozen for the determination of sulfate and other major anions by ion chromatography (IC; Metrosep A Supp. 5 column; Metrohm, Switzerland). Further aliquots were acidified (1% v/v of concentrated HCl) for the determination of major cations by inductively-coupled plasma optical emission spectrometry (ICP-OES; Vista MPX; Varian, USA).

The soil material from the three replicates per sampling time was combined, homogenized, divided into subsamples, sealed in gas tight bags and frozen in liquid nitrogen. A subsample was freeze-dried and stored in the glovebox for analysis of total elemental composition by energy
dispersive X-ray fluorescence spectrometry (XRF; X-Lab 2000; Spectro, Germany) and Cd speciation by X-ray absorption spectroscopy (XAS). For selected soil samples (2d EQ, 10d RED, 40d RED, 28d REXO) a subsample of the frozen soil was allowed to thaw in the glovebox and used immediately as wet paste for two-step sequential extraction. Soluble and exchangeable Cd ("mobile Cd fraction") was extracted with an unbuffered 0.1 M CaCl$_2$ solution (McGrath and Cegarra, 1992). Specifically adsorbed Cd and Cd in carbonates and other minerals labile at pH 5 ("easily mobilizable Cd fraction") were subsequently extracted using 1 M sodium acetate adjusted to pH 5 (Tessier et al., 1979). Extractions were performed in the glovebox using deoxygenated solutions to prevent sample oxidation (Rapin et al., 1986). 1 g of soil (dry-weight basis) was extracted in triplicate with 25 mL extractant by shaking suspensions end-over-end at room temperature for 24 h. Suspensions were centrifuged (15 min at 3566 g) and the supernatants were filtered through 0.45-μm nylon filters (Opti-Flow; Wicom, Germany), acidified (1% v/v concentrated HCl), and analyzed by ICP-OES using multi-element standards with the matrix of the respective extract. Between the two steps the soil residuum was washed once to prevent carry-over of residual solution into the next step.

### 3.2.4. X-ray absorption spectroscopy and data analysis

The speciation of Cd in selected soil samples from all incubation series (2d EQ, 20d RED (or 5d RED for MS-LCu), 40d RED, 28d REXO) was analyzed by Cd K-edge extended X-ray absorption fine structure (EXAFS) and X-ray absorption near edge structure (XANES) spectroscopy. The samples were ground, pressed into pellets and placed between Kapton® tape under N$_2$ atmosphere and kept anoxic until the measurement. Measurements were performed at the SuperXAS (X10DA) beamline at the Swiss Light Source (SLS, Paul Scherrer Institute, Villigen, Switzerland) equipped with two Pt-coated Si mirrors for vertical collimation and beam focusing and a double crystal Si(311) monochromator for energy selection. For energy calibration, the first inflection of the absorption $K$-edge of a metallic Cd foil was set to 26711 eV. For data collection, the samples were cooled to 100 K using a N$_2$ gas stream (Cryojet, Oxford Instruments, UK). Spectra of soil samples were recorded in fluorescence mode using a 13-element Ge solid state detector. Spectra of the following reference compounds were recorded in transmission or
fluorescence mode depending on Cd content (labels in brackets): Cd-carbonate (CdCO$_3$), Cd-nitrate (Cd(NO$_3$)$_2$), Cd sorbed to montmorillonite, goethite or carboxyl resin BioRex$^\circledR$ (Cd-clay, Cd-goethite, Cd-carboxyl), Cd sorbed on thiol resin GT74$^\circledR$ at pH 3 and pH 9 (Cd-thiol (pH3), Cd-thiol (pH9)), Cd complexed by soil humic acid (Cd-humic) and Cd-sulfide (CdS). Details on the source or synthesis of reference compounds and structural parameters derived from shell-fits to their EXAFS spectra are given in the Appendix B.1.

All spectra were processed using the analysis program Athena (Ravel and Newville, 2005). $E_0$ was fixed to 26711 eV. For the extraction of the EXAFS data, the spectra were normalized by subtracting a first-order polynomial function fit to the pre-edge region (−120 to −50 eV) and subsequently dividing by a second-order polynomial fit to the post-edge region (50 to 420 eV). A background spline was adjusted using the Autobk algorithm ($R_{bkg}=1.1$; $k$-weight = 3; $k$-range 0.5–10.5 Å$^{-1}$).

Based on principal component analysis of the sample EXAFS spectra, target transform testing of all reference spectra, and preliminary EXAFS LCF analysis (Appendix B.1), the reference spectra Cd-carboxyl, Cd-thiol (pH9) and CdS were selected for LCF analysis of the $k^3$-weighted soil EXAFS spectra ($k$-range 2.5 to 9.2 Å$^{-1}$; sum of fractions constrained to 100%). Using the same three references, also the XANES spectra were analyzed by LCF, including seven additional soil XANES spectra, three of which were recorded at the Dutch Belgian Beamline (DUBBLE) at the European Synchrotron Radiation Facility (ESRF, Grenoble, France) (Appendix B.4). Furthermore, the soil EXAFS spectra were also complementarily analyzed by shell-fitting (Appendix B.4).

### 3.3. Results and Discussion

#### 3.3.1. Dynamics of Eh, pH and major dissolved species

The dynamics of Eh, pH, dissolved Fe and Mn (Appendix B Figure B.2) and dissolved sulfate (Figure 3.1) during soil reduction followed similar trends in all four treatments and reflected the typical sequence of microbial respiration of different electron acceptors upon depletion of O$_2$ (i.e., denitrification, reductive dissolution of Mn(III/IV)- and Fe(III)-(oxyhydr)oxides, sulfate reduction and methanogenesis) (Ponnamperuma, 1972; Kirk, 2004; Koegel-Knabner et al., 2010), as described in detail previously for the Cu-spiked series (Fulda et al., 2013). Briefly, $E_h$
decreased rapidly after O\textsubscript{2} exclusion, and stabilized between -110 and -10 mV after 30 days of soil flooding. In parallel, solution pH increased slightly from ~6.6 to 7.1 during the reduction period. Dissolved Mn and Fe started to increase between day 1 and 5, reflecting the onset of microbial reduction of Mn(III/IV)- and Fe(III)-(oxyhydr)oxides. As indicated by decreasing dissolved sulfate (Figure 3.1a-d), major microbial sulfate reduction started after 5 days (MS-LCu), 10 days (LS-HCu), and 15 days (MS- and HS-HCu), respectively. In the MS-LCu series without spiked Cu, sulfate reduction was completed after 15 days, whereas complete sulfate consumption in the Cu-spiked series was only observed after 30 days of flooding. This difference between incubations without and with spiked Cu indicated that sulfate reducing bacteria in the Cu-spiked treatments were affected by Cu toxicity (Jin et al., 2007; Fulda et al., 2013). However, Cu spiking only retarded the onset of sulfidogenesis but did not prevent complete sulfate reduction within 40 days of soil flooding. Therefore, differences in Cd solubility and solid-phase speciation between the four treatments after 40 days of reduction were considered to be independent of Cu toxicity but related to the amounts of reducible sulfate and chalcophile Cu, competing with Cd for precipitation with sulfide. Note, that sulfide formed during soil reduction exceeded the initial amount of reducible sulfate due to minor mineralization of organic S (Fulda et al., 2013). This was quantitatively relevant in the LS-HCu series, where chromium(II) reducible sulfur (CRS) extraction suggested the formation 0.5 mmol kg\textsuperscript{-1} sulfide within 40 days of reduction (Fulda et al., 2013). Thus, also in the LS-HCu series, sulfide at the end of the reduction period exceeded the amount of Cd by factor 2.5, but was still ~9 times lower than (Cd+Cu).

After aeration of the soil suspensions, dissolved Fe and Mn decreased rapidly and were removed from solution within two days, indicating the oxidation of Fe(II) and Mn(II) and precipitation of Fe/Mn-(oxyhydr)oxides (Kirk, 2004) (Appendix B Figure B.2c-d). Dissolved sulfate, on the other hand, increased again due to oxidative dissolution of metal-sulfides (Figure 3.1a-d). After 28 days of soil reoxidation, dissolved sulfate in some treatments significantly exceeded the concentrations before soil reduction, pointing to concomitant mineralization of organic S upon aeration (Fulda et al., 2013). The steep increase of solution pH to about 8.4 within two days of reoxidation (Appendix B Figure B.2b) was attributed to the rapid release of CO\textsubscript{2} accumulated
during soil reduction (Fulda et al., 2013). Subsequently, solution pH gradually decreased over 28 days of reoxidation to values slightly higher than before soil reduction.

### 3.3.2. Dynamics of dissolved Cd and changes in Cd extractability

Changes in dissolved Cd during soil reduction and reoxidation in comparison to changes in dissolved sulfate and concomitant changes in extractable fractions of Cd are depicted in Figure 3.1. After the 2-day equilibration phase, less than 0.5% of the spiked Cd (and less than 0.1% of the spiked Cu) remained dissolved. At the early stages of soil reduction, the concentrations of dissolved Cd slightly increased in all Cu-spiked series (Figure 3.1a-c). This initial Cd release was attributed to the reductive dissolution of Fe(III)- and Mn(III/IV)-(oxyhydr)oxide sorbent phases and the resulting increase in dissolved divalent cations that compete with Cd for sorption sites (Cornu et al., 2007; Weber et al., 2009a; Hofacker et al., 2013; Huang et al., 2013). The more pronounced initial increase of dissolved Cd in the MS-LCu series (Figure 3.1d) was probably due to the faster microbial respiration of Mn(III/IV)- and Fe(III)-(oxyhydr)oxides in the absence of spiked Cu (Appendix B Figure B.2c-d). The susceptibility of Cd to displacement by competitively adsorbing cations during initial soil reduction was in line with the high initial Cd extractability. After the 2-day equilibration period, more than 70% of the soil Cd in the Cu spiked series was mobile (CaCl₂-extractable) and about 25% easily mobilizable (Na-acetate-extractable) (Figure 3.1e-h).

With ongoing soil reduction, dissolved Cd concentrations decreased in all treatments in parallel to dissolved sulfate. Comparing the three Cu-spiked series (Figure 3.1a-c), the decrease in dissolved Cd was fastest in the high sulfate series (HS-HCu), where Cd solution concentrations decreased by 60 nmol L⁻¹ d⁻¹, reaching values below the detection limit (5 nmol L⁻¹) after 20 days. In the medium-sulfate series (MS-HCu), dissolved Cd decreased with a rate of 43 nmol L⁻¹ d⁻¹ and reached values below detection limit after about 30 days. In the low-sulfate series (LS-HCu), Cd decreased gradually over the entire reduction period at a low rate of 10 nmol L⁻¹ d⁻¹ but was still measurable after 40 days of reduction. The nearly concomitant decrease of dissolved Cd and sulfate and the faster decrease of dissolved Cd at higher initial
Figure 3.1. Dissolved Cd and sulfate concentrations (left panels) and Cd extractability (right panels) during soil reduction and subsequent reoxidation. The respective periods of major sulfate reduction are marked as gray shaded areas in the left panels. Error bars in the left panels indicate the standard deviation of experimental triplicates. Error bars in the right panels indicate the standard deviation of three individual extractions. Extracted fractions of Cd are reported as percentage of total Cd determined by XRF (values in Appendix B Table B.4). (a, e) low-sulfate series with Cd and Cu spike (LS-HCu); (b, f) medium-sulfate series with Cd and Cu spike (MS-HCu); (c, g) high-sulfate series with Cd and Cu spike (HS-HCu); (d, h) medium-sulfate series with Cd spike (MS-LCu). Sulfate data for the low-, medium- and high-sulfate series with Cd and Cu spike are from Fulda et al. (2013).

Sulfate content pointed to the formation of Cd-sulfide during the sulfate reduction period, as also suggested in previous macroscopic studies on Cd solubility in wetland soils (Cornu et al., 2007; de Livera et al., 2011; Huang et al., 2013). Notably, in the MS- and HS-HCu series dissolved Cd started to decrease slightly before the onset of major sulfate reduction. This suggested that already minimal initial sulfate reduction (Fulda et al., 2013) may have led to Cd-sulfide formation, taking into account that initial dissolved sulfate concentrations were 2 to 4 orders of magnitude higher than dissolved Cd concentrations. In the medium-sulfate series without Cu
spike (MS-LCu, Figure 3.1d), Cd removal from solution started already after the first day of incubation, in line with the earlier start of sulfate reduction. The Cd decrease rate of 88 nmol L\(^{-1}\) d\(^{-1}\) was, however, about twice as high as in the corresponding medium-sulfate series with Cu spike (MS-HCu), and Cd was completely removed from solution already after 10 days. The acceleration of Cd removal from solution in the absence of Cu suggests that Cu competed with Cd for sulfide during metal-sulfide precipitation. The dependency between the decrease of dissolved Cd, sulfate availability and content of competing metals is supported by the good correlation between the Cd decrease rates of the four treatments and the molar ratio of initial soil sulfate over the sum of soil Cu and Cd contents (\(R^2 = 0.92\); Appendix B Figure B.3). Changes in Cd extractability during reduction reflected the observed Cd solution dynamics in the four treatments. In the MS-LCu series (Figure 3.1h), Cd was completely removed from the mobile fraction within the first 10 days and only 42% remained Na-acetate-extractable, which was in line with the faster decrease in Cd solubility and the assumed enhanced precipitation of Cd-sulfide in the absence of Cu. In contrast, in the Cu spiked series (Figure 3.1e-g), Cd remained in the mobile and easily mobilizable fraction during the first 10 days of reduction. The slight increase of Na-acetate-extractable Cd at the expense of CaCl\(_2\)-extractable Cd, as observed in all series, may be explained by stronger Cd adsorption on mineral surfaces as solution pH increased from 6.6 to 7.1 (Fischer et al., 2007; Saeki and Kunito, 2012). After 40 days of reduction, more than 91% of the Cd in the low-sulfate series (LS-HCu) remained extractable, in contrast to the medium- and high-sulfate series, where extractable Cd decreased to 18% (MS-HCu) and 5% (HS-HCu), respectively. In the medium-sulfate series without Cu spike (MS-LCu), Cd became non-mobilizable during soil reduction.

During reoxidation, dissolved Cd slightly increased, which we attributed to oxidative dissolution of metal-sulfides. Over 4 weeks of reoxidation, however, Cd concentrations remained below the initial values prior to soil reduction (Figure 3.1a-d). This may be due to effective readsorption of Cd on freshly precipitated Fe- and Mn-oxides (Calmano et al., 1994), especially at the relatively high pH values during the initial stage of soil reoxidation. Notably, in all Cu-spiked series Cd concentration slightly increased after 2 weeks concomitant to a decrease in dissolved Cu concentrations (Appendix B Figure B.9), which pointed to a competition between Cu and Cd for
3.3 Results and Discussion

Within 28 days of soil reoxidation, Cd was completely repartitioned into the mobile and easily mobilizable fractions (Figure 3.1e-h). However, the easily mobilizable (Na-acetate-extractable) Cd fraction remained higher than after the 2-day equilibration period, in line with the lower dissolved Cd levels.

3.3.3. Changes in Cd speciation by X-ray absorption spectroscopy

Although the observed changes in Cd solubility and extractability were in good agreement with expected trends, these macroscopic results provided no molecular-level insights into Cd speciation changes during soil reduction and reoxidation. We therefore used Cd K-edge X-ray absorption spectroscopy to obtain direct information on solid-phase Cd speciation changes, an approach that has not previously been applied to Cd speciation at such low Cd levels in soils. Figure 3.2a-d shows the $k^3$-weighted EXAFS spectra of selected soil samples, and their Fourier-transformed EXAFS magnitudes in comparison to the spectra of three Cd reference compounds (Cd-carboxyl, Cd-thiol (pH9) and CdS). The positions of the first-shell peaks in the Fourier-transformed EXAFS spectra of Cd-carboxyl and CdS reflect the difference between first-shell O at ~2.3 Å and first-shell S at ~2.5 Å (Appendix B Figure B.1). Accordingly, visual inspection of the Fourier-transformed sample EXAFS spectra indicated a shift from dominantly O-coordinated Cd in the 2-day equilibrated samples to dominantly S-coordinated Cd after the 40-day reduction period and back to dominantly O-coordination Cd after the 28-day reoxidation period (Figure 3.2d).

To further evaluate these trends, we analyzed the sample EXAFS spectra by linear combination fitting (LCF) using Cd-carboxyl, Cd-thiol (pH9) and CdS as references (LCF spectra in Figure 3.2b and 2d, LCF fractions in Figures 3.2e and Appendix B Table B.5). The LCF analysis of the sample EXAFS spectra was complemented and supported by shell-fits and by LCF analysis of the corresponding XANES spectra (Appendix B.4). In the EXAFS LCF analysis Cd-carboxyl served as a proxy for O-coordinated Cd adsorbed to natural organic matter, clay minerals, and metal oxides (Appendix B.1). According to shell-fit results (Appendix B Table B.1), Cd in these references is coordinated by ~6 O atoms at a distance of 2.27–2.30 Å. In the crystalline CdS reference, Cd is coordinated by 4 first-shell S atoms at a distance of 2.52 Å and 12 second-shell Cd atoms at
~4.1 Å (Appendix B Table B.1). The Cd-thiol(pH9) reference is characterized by a similar first-shell S coordination (~3.2 S at 2.52 Å) as crystalline CdS, but with a minor contribution from first-shell O (~1.6 O at 2.32 Å) and without the contribution from second-shell Cd atoms (Appendix B Table B.1). Second-shell Cd-Cd contribution may be strongly reduced or even absent in freshly-precipitated nanoparticulate CdS (Rockenberger et al., 1997), which may also exhibit a low degree of crystallinity and some isomorphic replacement of S\textsuperscript{2-} by O\textsuperscript{2-} or OH\textsuperscript{-} (Daskalakis and Helz, 1992). Therefore, nanometer-sized and/or poorly crystalline Cd-sulfide may have been represented by a combination of the Cd-thiol (pH9) and CdS references in LCF analysis. In addition, the Cd-thiol (pH9) reference also served as a proxy for Cd complexed by reduced organic S groups of natural organic matter (Smith et al., 2002; Karlsson et al., 2005; Karlsson et al., 2007) or bacterial cell walls (Mishra et al., 2010), or Cd adsorbed to metal sulfides. The interpretation of the LCF results with respect to S-coordinated Cd therefore required the concomitant consideration of the chemical extraction data. As metal binding to thiolate ligands is strongly pH-dependent, with low pH favoring the displacement of metal ions (Steffens, 1990), we assumed thiol-bound Cd in our samples to be Na-acetate-extractable, while Cd-sulfide phases are expected to be non-extractable.

LCF analysis of three 2-day equilibrated soil samples on average returned 76% Cd-carboxyl and 24% Cd-thiol (Figure 3.2e and Appendix B Table B.5). These fractions compared to ~75% CaCl\textsubscript{2}- and ~25% Na-acetate-extractable soil Cd in the 2-day equilibrated samples, which suggested the presence of thiol-bound Cd. Over the course of soil reduction, the S-coordinated Cd fractions increased (Figure 3.2) as dissolved Cd and extractable Cd decreased (Figure 3.1). Accordingly, LCF analysis of a larger set of sample XANES spectra revealed a close linear relation between increasing S-coordinated Cd and decreasing dissolved Cd (R\textsuperscript{2} = 0.87; Appendix B Figure B.8b). In combination with the decrease of extractable Cd fractions, these trends suggested that Cd sequestration into less soluble form was dominantly due to formation of Cd-sulfide precipitates. After 40 days of soil reduction, the CdS and Cd-thiol references accounted for 100% of the total Cd in the MS-LCu, HS-HCu, and MS-Cu series and for 90% of the total soil Cd in the LS-HCu series. The highest CdS fraction was found for the MS-LCu (69%) and the HS-HCu (64%) series, followed by the MS-HCu (51%) and LS-HCu (30%) series. In shell-fits, this trend was reflected by
3.3 Results and Discussion

Figure 3.2. (a,b) $k^3$-weighted EXAFS spectra and (c,d) corresponding Fourier-transform magnitudes ($k$-range 2.5–9 Å$^{-1}$, Kaiser-Bessel $dk = 2.5$ Å$^{-1}$) of Cd reference compounds (upper panels) and samples (lower panels, gray lines). (e) Cd speciation based on EXAFS LCF (values in Appendix B Table B.5). Fit spectra for EXAFS LCF are drawn as dotted red lines in panels (b) and (d).
decreasing second-shell Cd-Cd (from CdS) and increasing first-shell Cd-O coordination with decreasing (Cd+Cu)/sulfate ratio (see Appendix B Table B.7). The fact that Cd in the 40-days reduced sample from the MS-LCu series with the lowest (Cd+Cu)/sulfate ratio was neither CaCl$_2$-nor Na-acetate-extractable (Figure 3.1h) suggested a near-complete sequestration of Cd in a Cd-sulfide precipitate. The presence of 31% Cd-thiol derived from LCF analysis was therefore interpreted to account for a very small CdS crystallite size (Rockenberger et al., 1997) and/or low crystallinity (Daskalakis and Helz, 1992). From the shell-fit of this EXAFS spectrum, a low second-shell Cd coordination number of 4.2±1.7 was derived (Appendix B Table B.7), indicating a CdS crystallite size in the range of a few nm only (Rockenberger et al., 1997). In the LS-HCu series with the highest (Cd+Cu)/sulfate ratio, in contrast, most Cd in the 40-days reduced sample was still extractable (Figure 3.1e). This suggested that a significant portion of the LCF-derived Cd-thiol fraction in this sample effectively represented Cd bound by reduced organic S groups. An increase of the Cd-thiol fraction from the 2-day equilibrated to the 40-day reduced sample could be due to the growth of bacterial biomass and an increase in Cd-binding by sulphydryl-groups on bacterial cell-walls (Mishra et al., 2010) or due to increasing Cd complexation by thiol groups in humic acid newly formed by reaction with bisulfide (Hoffmann et al., 2012). Another explanation could be that some Cd adsorbed to the surface of newly-formed Cu$_x$S (Fulda et al., 2013) and was therefore more labile, similar to Cd adsorbed onto mackinawite (Coles et al., 2000). Based on the Cd speciation discussed for the series MS-LCu and LS-HCu, S-coordinated Cd in the intermediate series HS-HCu and MS-HCu represented mainly nanoparticulate Cd-sulfide and minor fractions of thiol-bound Cd, in line with Na-acetate-extractable Cd fractions (5% in HS-HCu; 18% in MS-HCu).

Upon reoxidation, the fraction of S-coordinated Cd species decreased rapidly in all treatments (Appendix B Table B.5 and Figure B.8a) and Cd speciation after 28 days of reoxidation closely resembled Cd speciation after soil equilibration (Figure 3.2e), irrespective of the different amounts of Cd-sulfide formed during soil reduction. The fast decrease in S-coordinated Cd suggests that all S-coordinated Cd species formed during soil reduction were readily oxidizible.
3.3 Results and Discussion

3.3.4. Solubility and solid-phase speciation of Cd as affected by reducible sulfate and Cu

Considering the Cu- and Cd-spiked series with varying reducible sulfate content (HS-HCu, MS-HCu, LS-HCu), our results clearly show that lower reducible sulfate contents may result in limited Cd-sulfide formation and thereby reduced Cd immobilization over the course of soil reduction. Furthermore, the comparison of the series without and with spiked Cu (MS-HCu, MS-LCu) clearly revealed that chalcophile Cu can reduce the extent of Cd-sulfide formation and Cd immobilization due to competitive metal sulfide precipitation. Nevertheless, even in the treatments with an excess of Cu over reducible sulfate (LS-HCu, MS-HCu), a significant fraction of the soil Cd was still sequestered into Cd-sulfide during soil flooding, despite the orders of magnitude lower stability of CdS than CuS, and the ~25-fold lower soil Cd than Cu contents. This can be attributed to the fact that Cd forms weaker complexes with NOM than Cu (Christl et al., 2001; Maurer et al., 2012) and also has a lower adsorption affinity for clay and oxide mineral surfaces (McBride, 1989; Fischer et al., 2007). This is reflected by the lower extractability of Cu (<1% CaCl$_2$-extractable) (Fulda et al., 2013) compared to Cd (>70% CaCl$_2$-extractable) in the equilibrated Cu- and Cd-spiked soils. Furthermore, part of the Cu(II) in the Cu(II)-spiked soils became rapidly reduced to Cu(I) after soil flooding, which resulted in strong Cu(I) binding to NOM and metallic Cu formation via disproportionation (Fulda et al., 2013). These transformation reactions may have further attenuated the competition of Cu with Cd for reaction with biogenic sulfide.

According to Cd EXAFS results, the Cd sulfide formed during soil reduction exhibited a very small crystallite size (few nm), but may also have been of poor crystallinity or partly even amorphous, as concluded for CuS in the Cu-spiked series based on Cu EXAFS data (Fulda et al., 2013). On the other hand, our Cd EXAFS results provided clear evidence for second-shell Cd-Cd coordination in Cd-sulfide, suggesting that at least a fraction of the Cd was present in a pure Cd-sulfide phase. Co-precipitation of Cu and Cd with sulfide was previously shown to lead to the formation of separate CdS and CuS phases rather than the formation of a mixed phase, which was attributed the large difference in the atomic radius between Cu and Cd (Tsamouras et al., 1999). Accordingly, the formation of Cu-rich Cd-containing metal sulfide nanoparticles with sizes in the range of a few tens of nanometers that we previously observed in a contaminated...
floodplain soil (Weber et al., 2009b; Hofacker et al., 2013) may result from the aggregation of extremely small but chemically pure Cu$_x$S and CdS crystallites. Notably, in the present study, Cd-sulfide readily dissolved during soil reoxidation whereas Cu$_x$S was more resistant to oxidative dissolution (Fulda et al., 2013), also hinting to the sequestration of Cu and Cd into separate but possibly co-aggregated metal-sulfides.

In contrast to a recent study on Cd speciation in a highly Cd contaminated alkaline paddy soil (Khaokaew et al., 2011), we did not observe any Cd-carbonate formation in our incubation experiment, most probably due to weakly acidic pH of the soil investigated here and the absence of calcite as a Cd-carbonate template (Stipp et al., 1992). Conversely, CdS formation in the study of Khaokaew et al. (2011) may have also been limited by the amounts of reducible sulfate.

### 3.3.5. Environmental implications

Our X-ray spectroscopic results clearly confirmed that decreasing Cd solubility and extractability during soil flooding can be related to formation of Cd-sulfide. Although, Cd-sulfide formation occurred even at Cu/sulfate ratios of >1, our results suggested that when the amount of Cd and competing chalcophile cations such as Cu significantly exceeds the amount reducible sulfate, Cd solubility and extractability may remain high even under anoxic conditions. Our results imply that rice growth under waterlogged conditions may not prevent Cd transfer into rice in multi-metal contaminated paddy soils with low sulfate content. Further, our results suggested that upon soil drainage Cd-sulfide phases formed during soil flooding periods readily oxidize, most likely due to their amorphous or nanoparticulate character. This has to be considered in the discussion of water management strategies like alternate soil drying and wetting where Cd-sulfide may be oxidized and therefore become phyto-available already during short drainage periods. Furthermore, the same dependency of Cd dynamics on reducible sulfate and Cu as described in this study may also control Cd mobility in periodically flooded riparian soils with low sulfate contents (Giblin and Wieder, 1992), where elevated Cd concentrations may also lead to enhanced Cd release into surface and groundwater resources.
Acknowledgements. We acknowledge the Swiss Light Source (SLS, Villigen, Switzerland) for allocation of beamtime at the SuperXAS beamline and the Dutch Belgian Beamline (DUBBLE, ESRF, Grenoble, France) for the allocation of CRG beamtime. Maarten Nachtegaal, Messaoud Harfouche and Xiaolan Liu (SLS) and Sergey Nikitenko (ESRF) are thanked for their support during XAS data collection. From ETH Zurich, we thank Anke Hofacker for her help with XAS measurements, Kurt Barmettler for technical and analytical support and Katrin Ehler for conducting parts of the incubation experiment. Jessica Dittmar is acknowledged for providing and characterizing the soil material used in this study. This research was financially supported by the Swiss National Science Foundation under grant No. 200021-117933 and No. 200020-135338.
3.4. References


4. Copper redox transformation and complexation by reduced and oxidized soil humic acid: 1. X-ray absorption spectroscopy study

This chapter is the first part of two companion articles that has been submitted with minor modifications for publication in Environmental Science and Technology: Fulda, B., Voegelin A., Maurer F., Kretzschmar R. Copper redox transformation and complexation by reduced and oxidized soil humic acid: 1. X-ray absorption spectroscopy study.

Abstract

Natural organic matter (NOM) exerts strong influence on copper speciation and bioavailability in soils and aquatic systems. In redox-dynamic environments, electron transfer reactions between copper and redox-active moieties of NOM may trigger Cu(I) and Cu(0) formation. To date, little is known about Cu-NOM redox interactions and Cu(I) binding to NOM. Here, we present X-ray absorption spectroscopy results on copper redox transformations upon addition of Cu(II) or Cu(I) to untreated and electrochemically reduced soil humic acid (HA) under oxic and anoxic conditions. Both untreated and reduced HA mediated copper redox transformations. Under anoxic conditions, Cu(II)-HA complexes prevailed, but smaller fractions of copper were also stabilized as Cu(I)-HA in a 3-
to 4-fold coordination. Our results show that Cu-HA redox interactions are strongly affected by binding of Cu(II) and Cu(I) to HA and that HA contributes to the stabilization of Cu(I) against disproportionation.

### 4.1. Introduction

Copper (Cu) is an essential trace element for most living organisms as it is important for the viability of a variety of proteins and enzymes that carry out fundamental biological functions (Rubino and Franz, 2012). In terrestrial and aquatic systems, copper is involved in important biogeochemical cycles such as methane oxidation and denitrification (Averill, 1996; Semrau et al., 2010). However, elevated copper concentrations are toxic to most bacteria (Baath, 1989) and plants (Fernandes and Henriques, 1991) as free copper ions can catalyze the formation of reactive oxygen species that cause oxidative cell damage (Gaetke and Chow, 2003). The bioavailability of copper is therefore an important factor for ecosystem functioning.

Strong copper binding by natural organic matter (NOM) is one of the key factors controlling copper bioavailability in soils (McBride et al., 1997). Binding of Cu(II) to NOM has been intensively studied over the past decades (Xia et al., 1997; Korshin et al., 1998; Frenkel et al., 2000; Karlsson et al., 2006; Manceau and Matynia, 2010). Five- to six-membered ring chelates formed by closely-spaced carboxyl and hydroxyl groups were shown to be the dominant form of Cu(II)-NOM complexes (Manceau and Matynia, 2010). At low Cu-to-C ratios (<0.005), nitrogen-containing functional groups are most likely also involved in Cu(II) complexation (Frenkel et al., 2000).

Association of copper with NOM is especially important in NOM-rich soils like peat or wetland soils where periods of high water levels can retard NOM decomposition (Sahrawat, 2004). In an early study, metallic copper accumulation was observed in a peat bog (Lovering, 1927; Lett and Fletcher, 1980) and attributed to Cu(II) reduction by bacterial metabolites (Lovering, 1927). Recent studies on contaminated soils showed that shortly after flooding, Cu(II) was reduced to Cu(I) and even Cu(0) (Weber et al., 2009; Hofacker et al., 2013), which was attributed to Cu(II) reduction in the course of microbial detoxification. In another study, Cu(II) was shown to be reduced by redox-active moieties of NOM (Pham et al., 2012), as reported previously for other
redox-active elements like Fe, Hg, Ag, and As (Palmer et al., 2006; Bauer and Kappler, 2009; Gu et al., 2011; Maurer et al., 2012). Untreated Suwannee River fulvic acid was able to reduce Cu(II) to Cu(I) even under oxic conditions (Pham et al., 2012). Since humic substances are known to reversibly accept electrons during microbial respiration (Lovley et al., 1996; Scott et al., 1998), the electron donating capacity (EDC) of NOM is expected to be even higher under reducing conditions which may promote metallic Cu(0) formation.

Free aqueous Cu\(^{+}\) ions are considered to be rather unstable due to rapid oxidation and disproportionation to Cu(0) and Cu\(^{2+}\) (Fenwick, 1926; Sharma and Millero, 1988). However, Cu(I) can be stabilized by complexation as shown for chloride (Yuan et al., 2012) and natural organic ligands in estuarine systems (Leal and van den Berg, 1998). Compared to Cu(II), binding of Cu(I) by organic ligands is expected to be significantly different due to its higher thiophilicity, suggesting that Cu(I) forms strong complexes with reduced organic sulfur (Smith et al., 2002). Thiol-containing ligands are also thought to stabilize Cu(I) in oxic estuarine waters (Leal and van den Berg, 1998; Laglera and van den Berg, 2003). Reduced sulfur groups (thiols, thioethers, organic disulfides) comprise up to 70% of total sulfur in humic substances (Xia et al., 1998; Hutchison et al., 2001; Zhao et al., 2006; Prietzel et al., 2007) but due to low total sulfur contents (0.03–0.8 mol kg\(^{-1}\)) (Tipping, 2002), they are generally less abundant than nitrogen-containing moieties. Therefore, heterocyclic aromatic compounds like purines and imidazoles, which represent 10–41% of total nitrogen (Thorn and Cox, 2009) amounting to 0.5–4.5 mol kg\(^{-1}\), may also be relevant for Cu(I) complexation, similar to microbial cuproproteines (Rubino and Franz, 2012).

In contrast to the broad knowledge of Cu(II)-NOM binding under oxic conditions, studies on copper interaction with NOM under anoxic conditions elucidating both formation and binding of Cu(I) are lacking. We addressed this knowledge gap with a study, which comprises two parts. The aim of the first part, which is presented here, was to investigate redox transformations and complexation of Cu(II) and Cu(I) under both oxic and anoxic conditions using untreated and electrochemically reduced soil humic acid (HA) as a model for redox-reactive NOM. Two copper loadings were used to evaluate the potential role of low-abundant high-affinity Cu(I) binding sites in HA. Oxidation state and local coordination of copper bound to HA were determined with
X-ray absorption spectroscopy at the Cu K-edge. In the second part of this study (Maurer et al., 2013), we present binding isotherms for Cu$^{2+}$ and Cu$^+$ and show quantitatively how binding of copper to HA influences copper redox speciation in reduced and reoxidized HA solutions.

4.2. Materials and Methods

All solutions were prepared using ultrapure deionized water (Milli-Q®, 18 MΩ cm) and chemicals of at least analytical grade. For the preparation of anoxic solutions, water was purged with N$_2$ for at least 2 h. All anoxic work was performed under N$_2$ atmosphere (O$_2$ < 1 ppm) in a glovebox (Braun, Germany).

4.2.1. Humic acid solutions

A well characterized humic acid (HA) extracted from a humic Gleysol in northern Switzerland was used for this study (Christl et al., 2000). The HA solutions used for copper spike experiments were prepared in two redox treatments. Untreated HA (HA$_{untr}$) solution was prepared in the glovebox by diluting a HA stock solution to 3.8 g L$^{-1}$ (0.02 M NaCl, pH 6.24). Depending on the residence time in the glovebox, the redox potential ($E_h$) of this solution, as measured with a Pt-redox electrode (Metrohm 6.0351.100) in combination with a Ag/AgCl-reference electrode (Metrohm 6.0733.100), ranged from +50 to −10 mV (potentials against the standard hydrogen electrode). Reduced HA (HA$_{red}$) solution was prepared from untreated HA solutions by electrochemical reduction using a glassy carbon electrode (Aeschbacher et al., 2010) following the method described by Maurer et al. (2010). During reduction, ~0.55 mol kg$^{-1}$ electrons were transferred to the HA (Maurer et al., 2010). The HA$_{red}$ solution exhibited a pH of 6.81 and an $E_h$ of −0.14 V before copper addition.

4.2.2. Samples for XAS analysis

For spectroscopic analysis, HA$_{untr}$ and HA$_{red}$ were reacted with Cu(II) and Cu(I) in the glovebox as follows. Cu(II)-stock and Cu(I)-stock solutions were prepared by dissolving CuCl$_2$ (Merck, p.a.) or CuCl (Merck, p.a.) and NaCl (Merck, p.a.) in 2 M HCl (Merck, p.a.) and diluting to final
concentrations of 5 mM CuCl₂/CuCl, 0.1 M HCl and 0.5 M NaCl. Cu-stock solutions and 0.1 M NaOH (Sigma-Aldrich, p.a.) were iteratively added to HA_untr and HA_red in the glovebox to achieve copper loadings of 7.5 (low loading, L) and 150 mmol kg⁻¹ (high loading, H) while maintaining a pH between 4 and 7.5. Readjusted to a pH of 7.0, samples were equilibrated for one week either under anoxic conditions in the glovebox (subscript label anox) or purged with air for 15 min and kept under oxic conditions outside the glovebox (subscript label ox) to examine the effect of O₂. After equilibration, the oxic samples were transferred back into the glovebox and the pH of all samples was readjusted from ~6.7 to 7.0 using 0.1 M NaOH. Subsequently, the solutions were dried in the vacuum antechamber at $P < 0.01$ bar. Sample compositions and corresponding sample names are listed in Table 4.1.

### 4.2.3. X-ray absorption spectroscopy

Cu K-edge X-ray absorption near edge structure (XANES) and extended X-ray absorption fine-structure (EXAFS) spectra were collected at beamline 11-2 at the Stanford Synchrotron Radiation Lightsource (SSRL, Stanford, USA) and the XAS beamline at the Angströmquelle Karlsruhe (ANKA, Karlsruhe, Germany). Sample measurements were performed in fluorescence mode at 4 K (SSRL) or 15 K (ANKA) using closed cycle He-cryostats. Spectra of concentrated reference compounds (denotations in italics) were measured in transmission and fluorescence mode: Cu(II)-malate (Cu(C₄H₅O₅)₂(H₂O)₂); Cu(II)-carboxyl (Cu(II) sorbed to carboxyl resin BioRex®); Cu(I)-thiol (Cu(I) sorbed to thiol resin GT74®); Cu(I)-carb (CuCl reacted with sodium diethyldithiocarbamate); Cu metal (Cu foil). In addition, the following published spectra were used as references for data analysis: Cu(II)-CopC and Cu(I)-CopC (Cu(II) and Cu(I) complexed by Cu-trafficking protein CopC (Arnesano et al., 2003), spectra provided by Stefano Mangani, Università di Siena); Cu(I)-2S and Cu(I)-3S ([N(C₃H₇)₄]Cu(SC₁₀H₁₃)₂ and ([C₆H₅]₄P)₂Cu(SC₆H₅)₃ (Pufahl et al., 1997), spectra provided by James Penner-Hahn, University of Michigan); Cu(I)-mb (Cu(I) complexed by methanobactin (Hakemian et al., 2005), spectrum provided by Timothy Stemmler, Wayne State University); Cu(I)-OCl and Cu(I)-2Cl (linear Cu(H₂O)Cl and CuCl₂ complexes (Fulton et al., 2000), spectra provided by John Fulton, Pacific Northwest National
Table 4.1. Overview of samples and synthesis conditions. Samples were labeled according to the copper loading (L = low, i.e. 7.5 mmol kg\(^{-1}\) Cu; H = high, i.e. 150 mmol kg\(^{-1}\)), the oxidation state of spiked copper (2 = Cu(II); 1 = Cu(I)), the pretreatment of HA (untr = untreated; red = electrochemically reduced), and the equilibration condition (anox = anoxic, ox = oxic).

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<th>Label</th>
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<td></td>
<td>untreated HA</td>
<td>anoxic</td>
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<td>L1</td>
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</tbody>
</table>

\(^a\) HA composition: C 552 g kg\(^{-1}\), N 33 g kg\(^{-1}\), S 4 g kg\(^{-1}\) (Christl et al., 2000); \(^b\) molar Cu-to-organic C ratios: 0.0002 at 7.5 mmol kg\(^{-1}\) and 0.0033 at 150 mmol kg\(^{-1}\).

Laboratory); Cu(I)-3Cl\(^{-}\) (trigonal planar CuCl\(^{3-}\) complex (Brugger et al., 2007), spectrum provided by Barbara Etschmann, University of Adelaide).

The samples were analyzed by linear combination fitting (LCF) of the normalized XANES spectra (energy range 8968–9018 eV) and the \(k^3\)-weighted EXAFS spectra (\(k\)-range 2.5–10.3 Å\(^{-1}\)). Individual fractions were constrained to the range 0–1. For LCF analysis of the EXAFS spectra, the sum of all fitted fractions was constrained to 1. Using our set of 13 reference spectra, all possible one- to five-component fits were calculated. The best fit was chosen based on its normalized sum of squared residuals (NSSR = \(\sum^i (\text{data}_{\text{exp}} - \text{data}_{\text{fit}})^2 / \sum^i (\text{data}_{\text{exp}})^2\)). Starting from the best one-component fit (\(n = 1\)), the best \(n + 1\) component fit was only considered to be better than the best \(n\)-component fit if its NSSR was at least 10% (relative) lower (Jacquat et al., 2009). Additionally, shell-fit analysis of the \(k^3\)-weighted L1-red\(_{anox}\) and H1-untr\(_{anox}\) EXAFS spectra was performed in \(R\)-space using the software code Artemis (Ravel and Newville, 2005). Theoretical EXAFS phase-shift and amplitude functions for first-shell Cu-O and Cu-S paths were calculated with FEFF v.8.4 (Ankudinov et al., 1998) based on the structure of Cu(I)-ethylenethioacetate (Ogawa et al., 1982). Further details on spectra collection, data processing as well as synthesis and structure of reference compounds are given in Appendix C.1.
4.3 Results and Discussion

Copper redox speciation, coordination by HA, and formation of metallic Cu(0) after reaction of Cu(II) and Cu(I) with HA were investigated by with Cu $K$-edge XANES and EXAFS spectroscopy. The fractions of Cu(II), Cu(I), and Cu(0) derived from LCF analysis of the respective XANES and EXAFS spectra deviated by less than 10% (absolute) from each other (Appendix C Figure C.1). The types of references returning the best fits, however, varied between XANES and EXAFS LCF analysis and from sample to sample (Table 4.2 and Appendix C Table C.2). This is due to the fact that the XANES region is highly sensitive to copper oxidation state and coordination geometry but less to the types of coordinated atoms. In contrast, EXAFS signals mainly depend on the type, number, and distance of neighboring atoms. Therefore, redox interactions of copper with HA and changes in copper oxidation state will be discussed based on XANES LCF results and the copper bonding environment based on EXAFS LCF analysis and shell fitting combined with the information on coordination geometry derived from the XANES spectra.

4.3.1. Copper redox interactions and stabilization of copper oxidation state by soil HA

Figure 4.1a-c shows the Cu $K$-edge XANES spectra of copper spiked HA samples in comparison to the spectra of Cu(II) and Cu(I) reference compounds and Cu(0). XANES spectra exhibit pre-edge and edge features depending on copper oxidation states (Kau et al., 1987). The Cu(I) reference compounds had a characteristic pre-edge peak at 8982-8984 eV corresponding to 1s→4p transitions (Figure 4.1a). The absorption edge and maximum (white line) of Cu(II) compounds was shifted to higher energies and peaked at ~8996 eV corresponding to 1s→continuum transitions.

Figure 4.1d-e shows the copper redox speciation in the copper spiked HA samples based on XANES LCF analysis (values in Appendix C Table C.2). Addition of Cu(II) to untreated HA and subsequent equilibration in the presence of $O_2$ (L2- and H2-untr.ox) resulted in the predominant formation of Cu(II)-HA complexes, but also small amounts of Cu(I)-HA were formed (12% of total copper in L2-untr.ox). It has been shown previously that untreated humic acids have the ability to reduce redox-sensitive elements like Fe, Hg, Ag and As (Alberts et al., 1974; Palmer et al., 2006; Bauer and Kappler, 2009; Maurer et al., 2012). The electron donating capacity (EDC) of
untreated humic acids has been attributed to the presence of phenolic groups (Aeschbacher et al., 2012). For the untreated HA used here, an EDC of 0.13 mol kg\(^{-1}\) with respect to DCPIP (2,6-dichloro-phenol indophenol, \(E^\circ_{DPIP} = +0.217\) V) was determined at pH 7 (Maurer et al., 2010). The standard redox potential of the Cu\(^{2+}/\)Cu\(^+\) couple (+0.153 V) is lower than that of DCPIP. Therefore, Cu(II) is expected to acquire less electrons than DCPIP. Recently, Pham et al. (2012) reported rapid formation of Cu(I) upon reaction of Cu(II) with Suwannee river fulvic acid under oxic conditions, equivalent to the transfer of 0.06–0.1 mol kg\(^{-1}\) electrons, which is close to the EDC\(_{DCPIP}\) value determined for our untreated HA. However, in the presence of O\(_2\) additional oxidants formed during oxidation of fulvic acid by Cu(II) promoting the reoxidation of initially formed Cu(I) (Pham et al., 2012). Correspondingly, very small amounts of Cu(I) were present in L\(_2\)-untr\(_{ox}\) and H\(_2\)-untr\(_{ox}\) after one week of oxic equilibration. Compared to untreated HA, reduced HA is expected to donate more electrons to copper since ~0.55 mol kg\(^{-1}\) electrons were transferred to HA during electrochemical reduction (Maurer et al., 2010). Accordingly, 70–80% of Cu(II) spiked to reduced HA was reduced to Cu(I) (\(L_2\)-red\(_{anox}\): 6 mmol kg\(^{-1}\); \(H_2\)-red\(_{anox}\): 100 mmol kg\(^{-1}\)) under anoxic conditions. Furthermore, 16% of added Cu(II) was transformed to metallic copper at high copper loading (\(H_2\)-red\(_{anox}\)), while at low loading (\(L_2\)-red\(_{anox}\)), less than 4% of copper was transformed into Cu(0). The remaining amounts of Cu(II) indicate that parts of Cu(II) were strongly bound to the HA, which prevented complete Cu(II) reduction. This is in line with our companion study, where Cu(I) formation was strongly delayed at low loadings, which was attributed to the presence of a small fraction of strong binding sites for Cu\(^{2+}\) (Maurer et al., 2013). Similarly, the dependency of Cu(0) formation on copper loading suggests that at low copper loadings, formation of strong Cu(I)-HA complexes kept the free Cu\(^+\) concentration in solution low and thereby stabilized Cu(I) against further reduction at redox-active HA moieties and against disproportionation. This is in line with the Cu\(^+\) binding data in our companion study (Maurer et al., 2013). The dual competitive role of reduced HA for metal reduction and complexation has similarly been observed for mercury. Gu et al. (2011) found that at low loadings, Hg(II) reduction was outcompeted by strong Hg(II) complexation, while at high loadings Hg(II) was reduced to Hg(0) by reduced humic acid.
4.3 Results and Discussion

Figure 4.1. Normalized Cu K-edge XANES spectra: (a) Cu(II) and Cu(I) reference compounds and metallic Cu(0). (b-c) Sample spectra (solid line) with best LCF (symbols). (d-e) Redox speciation of copper in the presence of HA based on XANES LCF analysis (detailed results in Table C.2). Speciation includes Cu(II)-HA, Cu(I)-HA in 2-fold coordination (CN=2) and 3- to 4-fold coordination (CN=3–4), and metallic Cu(0).

At low copper loadings, no major differences in copper redox speciation was observed between reduced HA spiked with Cu(II) and Cu(I) (L2-red$_{anox}$ and L1-red$_{anox}$). At high copper loadings, considerably more Cu(0) was formed when Cu(I) was spiked to reduced HA (64% Cu(0) in H1-red$_{anox}$) compared to Cu(II) addition (16% Cu(0) in H2-red$_{anox}$). Considering that humic acids contain redox-active moieties that cover a wide range of redox potentials (Aeschbacher et al., 2011), it is likely that upon reduction of Cu(II) to Cu(I) the pool of electrons available from redox-active moieties was gradually consumed according to thermodynamic and kinetic favorability and, although only ~27% of the total EDC was used (Appendix C Table C.4), strong Cu(I) binding restricted further formation of Cu(0) in H2-red$_{anox}$. Correspondingly, we observed a significant increase in $E_h$ from ~200 mV to +25 mV upon titration of reduced HA with 180 mmol kg$^{-1}$ Cu(II) in our companion study (Maurer et al., 2013). Calculation of free Cu$^{+}$ and Cu$^{2+}$ activities in solution based on the Cu$^{2+}$ and Cu$^{+}$ binding isotherms presented in our
companion study (Maurer et al., 2013) and the equilibrium constant for copper disproportionation ($\log K_{\text{disp}} = 6.24$) (see Appendix C.4) revealed that all reduced HA samples equilibrated under anoxic conditions were close to saturation with respect to metallic copper (Appendix C Figure C.4). This suggested that in these samples equilibrium conditions were reached regarding the two competitive processes of electron transfer and copper binding. Whether the formation of Cu(0) at high copper loading was due to Cu(I) disproportionation (with cycling reduction of Cu(II) formed by disproportionation) or due to direct reduction of Cu(I) by HA cannot be determined unequivocally. But evidence for a direct electron transfer reaction between Cu(I) and HA is given by the absence of Cu(0) in the Cu(I) spiked untreated HA samples equilibrated under anoxic conditions (L1-untr$_{\text{anox}}$ and H1-untr$_{\text{anox}}$). If Cu(0) in H2-red$_{\text{anox}}$ and H1-red$_{\text{anox}}$ formed via disproportionation and depended only on the extent of Cu(I) stabilization by HA binding or chloro-complex formation, then Cu(0) formation would first of all be expected in the sample H1-untr$_{\text{anox}}$ as under moderate reducing conditions Cu(I) binds less strongly than under strongly reducing conditions (Maurer et al., 2013). The absence of Cu(0) in this sample consequently indicates that untreated HA was unable to reduce Cu(I) to Cu(0) under these conditions. This suggests that Cu(0) observed in HA$_{\text{red}}$ under anoxic conditions predominantly formed via direct Cu(I) reduction by the reduced HA rather than via disproportionation of free Cu$^{+}$, and that Cu(0) nanoparticles were stable during the 7-day equilibration only when solutions were close to Cu(0) saturation (additional TEM images see Appendix C.3).

It is noteworthy that only 62% of spiked Cu(I) in H1-untr$_{\text{anox}}$ and 28% of spiked Cu(I) in L1-untr$_{\text{anox}}$ persisted as Cu(I). This demonstrates that considerable fractions of added Cu(I) were oxidized to Cu(II) in the absence of O$_{2}$. Considering the high affinity of HA for Cu(II) binding under these redox conditions (Maurer et al., 2013) and the wide $E_h$ range covered by redox-active moieties of humic acids (Aeschbacher et al., 2011; Aeschbacher et al., 2012), it seems likely that Cu(I) added to untreated HA in the absence of O$_{2}$ can be partially oxidized and stabilized as Cu(II)-HA.

Despite electrochemical reduction, oxic equilibration of HA$_{\text{red}}$ resulted in a clear dominance of Cu(II)-HA (>90%), irrespective of copper loading and oxidation state of added copper. We
assume that copper was dominantly Cu(I) in these samples when brought into contact with O₂ (one hour after copper addition in the glovebox) because reduction of Cu(II) to Cu(I) by fulvic acid was shown to be a fast process, occurring on the timescale of minutes (Pham et al., 2012), while according to our companion study (Maurer et al., 2013) Cu(0) formation in presence of HA was apparently a fairly slow process. Our results show that most Cu(I)-HA complexes present under anoxic conditions were unstable under oxic conditions. Page et al. (2012) recently reported that oxygenation of a reduced humic acid solution in the dark led to the formation of reactive oxygen species, which may have acted as strong Cu(I) oxidants in our experiments. The remaining Cu(I) in aerated HA_red samples amounted to about 1 mmol kg⁻¹ at low copper loadings (L2/1-red_ox) and 8–9 mmol kg⁻¹ at high loadings (H2/1-red_ox). These amounts of Cu(I) stabilized by HA in the presence of O₂ closely match the findings of our companion study showing that untreated HA stabilized up to 10–15 mmol kg⁻¹ Cu(I) by complexation (Maurer et al., 2013).

From our spectroscopic results, we conclude that mutual electron transfer can occur between copper and HA. Under anoxic conditions, significant amounts of Cu(I)-HA complexes are formed. Depending on the copper loading, binding of Cu(I) to HA can effectively stabilize Cu(I) against further reduction and disproportionation, and to a minor extent, also against oxidation by O₂, implying that part of Cu(I) is very strongly bound to HA. To elucidate the stabilization of copper oxidation states in HA complexes, results on copper coordination will be presented and discussed in the following.

4.3.2. Coordination environments of Cu(I) and Cu(II)

Figure 4.2 illustrates the Cu K-edge EXAFS spectra and the magnitudes of corresponding Fourier transforms of copper reference compounds and copper-spiked HA. EXAFS spectra of samples containing mainly Cu(II), i.e. samples that were equilibrated under oxic conditions, were predominantly fitted by the Cu(II)-malate reference (Table 4.2), pointing to the formation of five-membered ring chelates reported in previous studies on Cu(II) binding by NOM (Karlsson et al., 2006; Manceau and Matynia, 2010). In contrast to the broad knowledge of Cu(II) complexation, the binding of Cu(I) by terrestrial NOM has not been investigated to date. In the
following section, we will therefore focus on the analysis of Cu(I) bonding environment and discuss coordination geometry and types of neighboring atoms in detail.

Linear combination fitting of the XANES spectra of samples with significant Cu(I) fractions suggested the presence of a mixture of 2-fold and 3- to 4-fold coordinated Cu(I) complexes (Figure 4.1b-e, Appendix C Figure C.2). These Cu(I) coordination geometries are distinguishable by their differences in the normalized absorption amplitude of the 1s→4p transition peak at 8982-8984 eV, which is most pronounced in the 2-fold near linearly-coordinated Cu(I) compounds, and the shape of the XANES spectra on the higher energy side of the 1s→4p transition peak (Kau et al., 1987; Pickering et al., 1993) (Figure 4.1a). Based on XANES LCF, 60–70% of the Cu(I) fraction in anoxic HA_red were fitted by 2-fold coordinated references (Figure 4.1d-e, Appendix C Table C.3). In anoxic HA_untr, the fraction of 2-fold coordinated Cu(I) was slightly lower and in the aerated samples, only 3- to 4-fold coordinated Cu(I) reference compounds appeared in the best XANES LCF.

Although EXAFS analysis in general provides sensitive information on the type of neighboring atoms, it should be noted that differing backscattering properties of the neighboring atoms are a prerequisite since EXAFS does not allow differentiating between atoms with similar atomic number due to their similar phase and amplitude functions. Consequently, Cu-O vs. Cu-N and Cu-S vs. Cu-Cl coordination cannot be distinguished unequivocally. Furthermore, high intensity of the EXAFS oscillations of metallic copper overlay with the oscillations of lighter neighboring atoms and complicate the analysis of Cu(I) coordination. Therefore, we will discuss the Cu(I) coordination environment with respect to the type of neighboring atoms based on the EXAFS spectra of samples which were dominated by Cu(I) and did not contain Cu(0).

The position of the major Fourier-transform peak in L2-red_{anox} and L1-red_{anox} spectra (80% Cu(I) in XANES LCF) pointed to the presence of S/Cl neighbors in the first coordination shell (Figure 4.2f). Compared to spectra where Cu(II) was the dominant oxidation state and the major Fourier-transform peak at ~1.5 Å (R+ΔR) corresponded to O/N atoms in the first coordination shell, the first shell peak in the L2-red_{anox} and L1-red_{anox} was shifted to the 1.5–2.2 Å R+ΔR-range, which indicates S/Cl neighboring atoms. This shift was also present, but less apparent in H1-untr_{anox} (62% Cu(I) in XANES LCF) (Figure 4.2d). Correspondingly, these spectra were accurately
4.3 Results and Discussion

Figure 4.2. (a-b) $k^3$-weighted EXAFS spectra and Fourier-transform magnitudes of Cu(II) and Cu(I) reference compounds and metallic Cu(0). (c-f) $k^3$-weighted EXAFS spectra and Fourier-transform magnitudes of sample spectra (solid line) and best LCF spectra (symbols). LCF results are listed in Table 4.2.

described by a linear combination of Cu(II)-O/N and Cu(I) references containing S/Cl atoms in the first coordination shell. In agreement with XANES LCF results on coordination geometry, Cu(I) speciation in the best EXAFS LCF was dominated by 2-fold coordinated Cu(I) exclusively represented by the linear Cu(I)-OCl reference (rather than the Cu(I)-2Cl or Cu(I)-2S references) (Table 4.2). The fraction of 3- and 4-fold coordinated Cu(I) was represented equally often by reference compounds with mixed O/N- and S-coordinated Cu(I) or exclusively S/Cl-coordinated Cu(I). The mixture of O/N and S/Cl in the first coordination shell caused substantial dampening or even extinction of the first-shell EXAFS oscillations in the higher $k$-range due to destructive interferences of O/N and S/Cl signals (Figure 4.2a), which is particularly evident in the L2-red$_{anox}$ and L1-red$_{anox}$ spectra (Figure 4.2c and e).
Table 4.2. Copper speciation based on LCF of $k^3$-weighted Cu K-edge EXAFS spectra. Individual fractions were constrained to the range 0-1 and the sum of all fitted fractions was constrained to 1. CN=2 and CN=3-4 refers to Cu(I) in 2-fold and 3- to 4-fold coordination.

<table>
<thead>
<tr>
<th></th>
<th>Cu(II)-O/N $^a$ (%)</th>
<th>Cu(I)-O/N+S/Cl $^b$ (CN = 2) (%)</th>
<th>Cu(I)-O/N+S/Cl $^c$ (CN = 3-4) (%)</th>
<th>Cu(I)-S/Cl $^d$ (CN = 3-4) (%)</th>
<th>Cu(0) (%)</th>
<th>NSSR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2-untr$_{ox}$</td>
<td>100 (mal)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>- 4.3</td>
<td></td>
</tr>
<tr>
<td>H1-untr$_{anox}$</td>
<td>34 (mal)</td>
<td>46 (OCI)</td>
<td>-</td>
<td>20 (3S)</td>
<td>-</td>
<td>3.4</td>
</tr>
<tr>
<td>H2-red$_{anox}$</td>
<td>22 (mal)</td>
<td>40 (OCI)</td>
<td>22 (copII)</td>
<td>-</td>
<td>17 0.4</td>
<td></td>
</tr>
<tr>
<td>H1-red$_{anox}$</td>
<td>11 (mal)</td>
<td>-</td>
<td>20 (mb)</td>
<td>-</td>
<td>70 0.3</td>
<td></td>
</tr>
<tr>
<td>H2-red$_{ox}$</td>
<td>99 (mal)</td>
<td>-</td>
<td>-</td>
<td>1.3</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>H1-red$_{ox}$</td>
<td>99 (mal)</td>
<td>-</td>
<td>-</td>
<td>1.2</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>L2-untr$_{ox}$</td>
<td>81 (mal)</td>
<td>19 (OCI)</td>
<td>-</td>
<td>-</td>
<td>- 3.9</td>
<td></td>
</tr>
<tr>
<td>L1-untr$_{anox}$</td>
<td>65 (mal)</td>
<td>35 (OCI)</td>
<td>-</td>
<td>-</td>
<td>- 8.1</td>
<td></td>
</tr>
<tr>
<td>L2-red$_{anox}$</td>
<td>13 (copII)</td>
<td>51 (OCI)</td>
<td>9.3 (copII)</td>
<td>27 (3S, carb)</td>
<td>- 2.2</td>
<td></td>
</tr>
<tr>
<td>L1-red$_{anox}$</td>
<td>16 (copII)</td>
<td>52 (OCI)</td>
<td>-</td>
<td>32 (carb, 3Cl)</td>
<td>- 2.3</td>
<td></td>
</tr>
<tr>
<td>L2-red$_{ox}$</td>
<td>99 (mal)</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>L1-red$_{ox}$</td>
<td>98 (mal)</td>
<td>-</td>
<td>-</td>
<td>1.6</td>
<td>3.9</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ (mal)=Cu(II)-malate, (copII)=Cu(I)-CopC protein; $^b$ (OCI)=Cu(I)-OCI; $^c$ (copII)=Cu(I)-CopC protein, (mb)=Cu(I)-methanobactin; $^d$ (3S)=Cu(I)-3S, (carb)=Cu(I)-carbamate

We investigated the mode of Cu(I)-HA coordination in more detail by fitting the $k^3$-weighted EXAFS spectra of the L1-red$_{anox}$ and H1-untr$_{anox}$ samples over the first coordination shell ($R+\Delta R$-range of 0.9–2.3 Å). We selected these samples because the content of Cu(0) was negligible and copper speciation was dominated by 80% (L1-red$_{anox}$) and 60% (H1-untr$_{anox}$) Cu(I) according to XANES LCF analysis (Figure 4.1, Appendix C Table C.2). The amplitude reduction factor ($S_0^2$), the energy-shift parameter ($\Delta E_0$) and the Debye Waller factor ($\sigma^2$) for Cu-O and Cu-S single scattering paths were derived from well-defined reference compounds (Cu(II)-malate, Cu(I)-2S and Cu(I)-3S). The fits and the corresponding EXAFS parameters are presented in Table 4.3 and Appendix C Figure C.6, respectively. The fitted distances for Cu-O and Cu-S paths in the reference compounds were in line with their theoretical crystallographic structure (Cocouvanis et al., 1980; Koch et al., 1984; Zhang, 2007). The shell-fit for L1-red$_{anox}$ suggested that copper was on average coordinated by 1.0 O/N atoms at a distance of 1.93 Å and by 1.1 S/Cl atoms at a distance of 2.25 Å. For the H1-untr$_{anox}$ sample Cu-O/N and Cu-S/Cl coordination numbers of 1.8 and 0.8 with distances of 1.92 Å and 2.22 Å were determined, respectively. Similar values were also
Table 4.3. Shell fitting results for $k^2$-weighted EXAFS spectra of model compounds and sample L1-red$_{anox}$ and H1-untr$_{anox}$. Shell fitting was performed in R-space over the first coordination shell ($k$-range 2.5–9.5, $dk = 2.5$; $R \pm \Delta R$-range 0.9–2.3).

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\sigma^2$</th>
<th>$\Delta E_0$ (eV)</th>
<th>path</th>
<th>CN</th>
<th>$R$ (Å)</th>
<th>$\sigma^2$ ($10^{-2}$ Å$^2$)</th>
<th>NSSR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)-malate</td>
<td>0.91 ±0.23</td>
<td>−3.38 ±3.30</td>
<td>Cu-O</td>
<td>4</td>
<td>1.94 ± 0.02</td>
<td>3.9 ± 2.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Cu(I)-2S</td>
<td>1.01 ±0.08</td>
<td>−5.83 ±0.99</td>
<td>Cu-S</td>
<td>2</td>
<td>2.15 ± 0.01</td>
<td>3.7 ± 0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Cu(I)-3S</td>
<td>1.02 ±0.10</td>
<td>−5.94 ±1.10</td>
<td>Cu-S</td>
<td>3</td>
<td>2.26 ± 0.01</td>
<td>10.4 ± 1.1</td>
<td>0.3</td>
</tr>
<tr>
<td>L1-red$_{anox}$</td>
<td>0.98$^b$</td>
<td>−5.22$^c$</td>
<td>Cu-O(N)$^f$</td>
<td>1.0 ± 0.2</td>
<td>1.93 ± 0.02</td>
<td>3.9$^d$</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cu-S(Cl)$^f$</td>
<td>1.1 ± 0.2</td>
<td>2.25 ± 0.01</td>
<td>7.1$^e$</td>
<td></td>
</tr>
<tr>
<td>H1-untr$_{anox}$</td>
<td>0.98$^b$</td>
<td>−5.22$^c$</td>
<td>Cu-O(N)$^f$</td>
<td>1.8 ± 0.3</td>
<td>1.92 ± 0.01</td>
<td>3.9$^d$</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cu-S(Cl)$^f$</td>
<td>0.8 ± 0.3</td>
<td>2.22 ± 0.02</td>
<td>7.1$^e$</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ fixed according to crystal structure; $^b$ average $\sigma^2$ of the reference compounds; $^c$ average $\Delta E_0$ of the reference compounds; $^d$ Debye-Waller factor of Cu-O in Cu(II)-malate; $^e$ average Debye-Waller factor of Cu-S in Cu(I)-2S and Cu(I)-3S; $^f$ Theoretical scattering paths for Cu-O and Cu-S were used for shell-fitting, but may also represent contributions from Cu-N and Cu-Cl, respectively, due to the similar EXAFS phase and amplitude functions of elements with similar atomic number.

obtained when the coordination numbers and interatomic distances were calculated from the EXAFS LCF fractions and the structural information of the included reference compounds (Appendix C Table C.6). The fitted Cu–S/Cl distance of 2.25 Å in L1-red$_{anox}$ sample matched the average distance known for 3-fold S-coordinated Cu(I) complexes (Sarret et al., 2010), rather than that of Cu–S/Cl distances in linear coordinated complexes, which is typically less than 2.20 Å (Helz et al., 1993; Poger et al., 2008). Considering that copper in sample L1-red$_{anox}$ was predominantly Cu(I), the low fitted oxygen and sulfur coordination numbers pointed to the presence of Cu(I) in both coordination geometries in line with XANES und EXAFS LCF results. Since we found in our companion study that more than 99% of total Cu(I) was strongly bound to the humic acid (Maurer et al., 2013), Cu(I) is expected to be coordinated to at least one ligating HA atom. Accordingly, 2-fold coordinated Cu(I) can be bound to HA in three possible ways: (i) via one O/N-group and coordinated with an additional chloride ion, (ii) via one S-group and coordinated with one H$_2$O or OH$^-$ molecule, or (iii) as bidentate complex bound to one O/N- and one S-group. In 3- and 4-fold coordinated geometry Cu(I) is most likely ligated by more than one HA atom. The binding environment may be similar to Cu(I) binding sites known for cuproproteines, where Cu(I) is bound via histidine (imidazol-N), cysteine (thiol, RS), and methionine (thioether, RSR) residues (Rubino and Franz, 2012). Such coordination...
environments are known to have a high formal Cu(II/I) redox potential keeping copper preferably in its Cu(I) oxidation state by destabilizing the Cu(II) oxidation state (Rorabacher, 2004). This effect has been found to be pronounced for tripodal ligands with increasing numbers of sulfur atoms, especially thioether donors (see Rorabacher and Schroeder (2007) and references therein). In this context it is noteworthy that 3- and 4-fold Cu(I) coordinated geometry was found to dominate Cu(I) speciation in HA equilibrated under oxic conditions, indicating a high stability of these Cu(I)-HA complexes against oxidation (Appendix C Table C.3). Concerning a contribution of oxygen donors, also binding of Cu(I) in thiolacetate-like bis-five-membered sulfhydryl/carboxyl chelates may represent a possible structure for Cu(I) complexation by NOM (Manceau and Matynia, 2010).

Concerning the type of Cu(I)-ligating atoms, the presence of up to 50–100 mmol kg\(^{-1}\) Cu(I) at high loadings (corresponding to XANES LCF analysis) would require an amount of 40–80 mmol kg\(^{-1}\) reduced S-groups based on the average Cu-S coordination number of 0.8 in the H1-untr\(_{anox}\) sample and assuming that coordination to Cl\(^{-}\) was negligible. However, S K-edge XANES spectroscopy of the HA used in this study showed that HA contained only 45 mmol kg\(^{-1}\) reduced organic sulfur (Maurer et al., 2012). Thus, even if all reduced sulfur is assumed to coordinate to Cu(I), the amount of reduced sulfur is not sufficient to completely account for Cu(I)-S/Cl coordination as derived from XAS analysis. This points to the presence of chloride in the first coordination sphere for binding of Cu(I) at high loading and suggests that a considerable fraction of Cu(I) was likely bound to the HA via O/N-groups. In contrast, at low loadings, the amount of reduced sulfur was sufficient to explain fitted amounts of S/Cl-coordinated Cu(I) (Cu(I)/S\(_{\text{red}}\) in L-HA\(_{\text{red}}\) = 0.13) considering the average Cu-S coordination of 1:1.

Among the reduced S-groups, thiolates (RS\(^{-}\)) may serve as high affinity binding sites for Cu\(^{+}\) as it was shown for other soft metal cations like Cd\(^{2+}\) and Hg\(^{2+}\) (Smith et al., 2002; Karlsson et al., 2005; Skyllberg et al., 2006). However, a previous study on competitive Cd\(^{2+}\) and Ca\(^{2+}\) binding to the HA used here revealed that at most 1 mmol kg\(^{-1}\) of reduced sulfur was present as thiols (Maurer et al., 2012). Consequently, thioether (RSH) and disulfide (RSSR) groups must also have contributed to Cu(I) complexation if Cu(I) in samples with low loadings was mainly ligated to sulfur groups. For cuproproteins, it is well known that methionine groups play an important role...
for Cu(I) binding (Rubino and Franz, 2012). Likewise, thioethers in HA may be involved in Cu(I) complexation. Disulfide groups were found to contribute to Hg(II) complexation by soil organic matter as inferred from the presence of a second shell S atom at higher distance in the coordination sphere of Hg(II) (Xia et al., 1999). Since the soft cations Hg\(^{2+}\) and Cu\(^{+}\) have a similar electronegativity (Stumm and Morgan, 1996), it seems likely that disulfide complexes may also be relevant for Cu(I) binding in HA.

### 4.3.3. Environmental implications

Our results highlight the dual role of humic acid as an electron donor/acceptor and sorbent for copper. The mutual electron transfer between copper and humic acid and the complexation of both Cu(I) and Cu(II) by functional groups of humic acids described in this study are expected to critically affect copper solubility in redox-dynamic environments. For example, in flooded contaminated soils with limited sulfate or prior to sulfate reduction, humic acid may effectively transfer electrons to Cu(II) and contribute to the stabilization of Cu(I) by its complexation at high-affinity sites, as suggested in earlier soil incubation studies (Hofacker et al., 2013). In sulfide-rich environments under strongly reducing conditions, Cu(I)-HA complexation may be less relevant as inorganic sulfide is expected to compete with organic ligands. However, for mercury it was shown that Hg(II)-thiol complexes and metacinnabar (β-HgS\(_{(s)}\)) can coexist in mixtures of organic soil and mackinawite (Skylberg and Drott, 2010), which may also apply for Cu(I)-HA complexes. On the other hand, humic acid has the potential for stabilizing small fractions of Cu(I) even upon soil reoxidation and to some extent also for reducing Cu(II) to Cu(I) under oxic conditions, an aspect that needs further investigation. Both mutual electron transfer between humic acid and copper and Cu(II) and Cu(I) binding by humic acid will influence the solubility of copper in equilibrium with humic acid. This aspect is further addressed in our companion study where we used potentiometric titrations and dialysis cell experiments to obtain binding isotherms for Cu\(^{2+}\) and Cu\(^{+}\) and gain quantitative information on the influence of copper binding by HA on copper redox speciation and solubility in the presence of reduced and reoxidized HA (Maurer et al., 2013).
Acknowledgements. Parts of this research were carried out at the Stanford Synchrotron Radiation Lightsource (SSRL), a Directorate of SLAC National Accelerator Laboratory and an Office of Science User Facility operated for the U.S. Department of Energy Office of Science by Stanford University. We are grateful to Joe Rogers for his support at beamline 11-2 (SSRL). We acknowledge the Angströmquelle Karlsruhe (ANKA, Karlsruhe, Germany) for the allocation of beamtime and thank Stephan Mangold for assistance at the XAS beamline and Anke Hofacker (ETH Zurich) who kindly helped during the measurements at ANKA. We are grateful to Ralf Kaegi (EAWAG) for performing the TEM analyses. From ETH Zurich, we also thank Kurt Barmettler for support in the laboratory and Martin Hoffmann for valuable discussions on data processing. This research was financially supported by the Swiss National Science Foundation under grant No. 200020-135338.
4.4 References


Fenwick, F. (1926) The equilibrium between cupric ion, cuprous ion and metallic copper. *JACS* 48, 860-870.


5. Conclusions

The results presented in this thesis demonstrate that the dynamics of Cu and Cd in soils with fluctuating redox conditions is a complex interplay of several processes that depend on the metal specific biogeochemical behavior and their competitive interaction, as well as on soil chemical parameters like the amount of reducible sulfate and the duration of the flooding period.

In the first part of this study (chapter 2) it was shown that Cu solubility and solid phase speciation during initial stages of soil flooding, i.e., prior to major sulfate reduction, is determined by a fast reduction of Cu(II) to Cu(I) and Cu(0). It was suggested that complexation of Cu(I) by reduced organic S groups of natural organic matter can be an important factor decreasing Cu solubility. These results were confirmed by a model experiment in the second part of this thesis (chapter 4), where it was shown that Cu(I) binding by humic acid likely involves the contribution of sulfur ligand groups, although binding by nitrogen ligand groups were quantitatively more important. The results further demonstrated that electron transfer processes between Cu(II) and reduced humic acid can be a possible pathway for the formation of Cu(I) and Cu(0) in soils. The precipitation of Cu(0) and complexation of Cu(I) by reduced organic S groups were also identified as key processes that determine Cu solubility in sulfate-limited soils during extended periods of soil flooding (chapter 2). In contrast, in soils with moderate to high reducible sulfate amounts Cu(0) is an intermediate phase and will be rapidly sulfidized during major sulfate reduction, leading to the formation of amorphous Cu-sulfide phases. The repartitioning of Cu into soluble and easily mobilizable fractions upon reoxidation was found to be closely related to the solid phase speciation during the previous reduction period. Cu(0) was shown to oxidize rapidly, while Cu-sulfide was stable over a period of up to two weeks.
Cd solution dynamics during initial soil reduction were controlled by the reductive dissolution of Fe- and Mn-(oxyhydr)oxides and competitive readsorption of Fe\(^{2+}\) and Mn\(^{2+}\) (chapter 3). Significant formation of nanometer-sized and/or poorly crystalline Cd-sulfide was observed in all series during major sulfate reduction, independent of the initial amount of reducible sulfate. However, the extent of Cd-sulfide precipitation and the decrease in Cd solubility and extractability was clearly related to the initial (Cd+Cu)/sulfate ratio. At ratios lower than unity Cd was almost complete transformed into poorly soluble sulfide phases, while at (Cd+Cu)/sulfate ratios much larger than unity Cd remained in a labile form. The weaker adsorption of Cd in soils compared to Cu and the specific redox transformation reactions of Cu (i.e., strong Cu(I) binding to NOM and metallic Cu formation) were identified as factors that attenuated the competition between Cu and Cd for reaction with biogenic sulfide. However, Cu- and Cd-sulfide precipitation may have been linked by the formation of mixed metal-sulfide phases, due to aggregation of small but chemically pure Cu\(_x\)S and CdS crystallites. In contrast to Cu-sulfide, Cd-sulfide was shown to be readily back transformed into soluble and easily extractable species in contact with O\(_2\). The extent of Cd-sulfide formation had therefore no visible influence on Cd remobilization upon reoxidation.

The observed Cu and Cd dynamics and identified key processes have strong implications for the risk assessment of trace metal mobilization in multi-metal contaminated riparian floodplain soils. In general, it was demonstrated that Cu solubility and extractability is strongly reduced during soil flooding, while elevated Cu concentrations in the porewater, and thereby potential release to surface waters and groundwater, can be expected during soil reoxidation. The potential risk of Cu export to adjacent water resources upon reoxidation may be higher in sulfate-limited soils, if the previous flooding period was not long enough or if soil reduction was not sufficient to reach major sulfate reduction, as in these cases Cu speciation during reduction is dominated by readily oxidizable Cu(0). The risk of Cd release is high in the initial stage of soil flooding until sulfate reducing conditions are reached and also during reoxidation due to the fast oxidative dissolution of Cd-sulfide. However, depending on the initial metal-to-sulfate ratio, contaminated floodplain soils may also act as a continuous source for Cd over the entire period of submergence. It should be noted that a soil incubation batch experiment, as conducted in this
thesis, does not allow to draw conclusions on the formation of colloids, which can potentially enhance metal mobilization during initial periods of soil flooding (Weber et al., 2009; Hofacker et al., 2013). Overall, the observed metal release patterns suggest that, in addition to the amount of reducible sulfate, also factors that influence sulfate reduction (e.g., flooding time and frequency, temperature) have to be considered for predicting metal mobility in multi-metal contaminated wetland soils.

The results of this thesis also have strong implications for the risk assessment of Cd soil-plant transfer in Cd contaminated paddy soils with low reducible sulfate content. There is consent that the phyto-availability of Cd can be controlled by flooding management, as it was shown that Cd concentrations in the grain of paddy rice grown under flooded conditions are significantly lower compared to aerobically grown rice (Bingham et al., 1976; Lee et al., 1996; Arao et al., 2009). However, this thesis demonstrated that in multi-metal contaminated paddy soils with very high metal-to-sulfate ratios Cd phyto-availability may remain high even under anoxic conditions. As Cd rarely occurs as a single contaminant in paddy soils, risk assessment should include analysis of the reducible sulfate amount in comparison to the total metal content of the soil. Furthermore, our results suggest that water management strategies like alternate soil drying and wetting may also be critical with respect to Cd uptake by rice plants, as Cd-sulfide may be readily oxidized and therefore become phyto-available even during short periods of soil drainage.

As outlined above, the observed redox-controlled transformations of Cu and Cd were strongly related to their metal specific biogeochemical behavior. In particular, the redox-active character of Cu, its high binding affinity in soils, and the high stability of Cu-sulfide stood in contrast to the weakly adsorbing Cd, which forms metal-sulfide of lower thermodynamic stability. This implies that, for example, Cd dynamics in sulfate limited soils may be significantly different from those observed in this thesis in the presence of metal contaminants other than Cu. Interactions between Pb-Cd and Zn-Cd may be especially relevant to study as they often co-occur in contaminated soils due to their common geochemical origin in Pb-Zn ores. In comparison to the Cu-Cd system, Pb has a similar sorption behavior in soils as Cu but has a lower metal-sulfide stability constant, which is close to that of Cd-sulfide. In contrast, the sorption affinity of Zn is similar to Cd, but Zn forms thermodynamically less stable sulfides than Cd (McBride, 1989).
Therefore, the influence of Pb and Zn on Cd dynamics may be different compared to Cu. A recent study addressed coupled Cd and Zn dynamics in a contaminated paddy soil (de Livera et al., 2011). Solution dynamics of Cd and Zn during reduction and oxidation under a low and high sulfate scenario suggested the formation of Cd- and Zn-sulfides. However, investigation of the concurrent changes in Cd and Zn solid phase speciation was lacking. It has to be considered, that additionally to their specific biogeochemical behavior, also the ratios at which different metals are present in multi-metal contaminated soils may significantly influence their coupled dynamics under changing redox conditions. For example, Pb and Zn are often dominant metal contaminants in polluted soils and especially Zn concentrations in mining/smelting affected soils typically exceed those of Cd at least by a factor of 100 (Chaney et al., 1996). Overall, the investigation of the behavior of various trace metals in binary systems can contribute to the understanding of their dynamics in sulfate limited multi-metal contaminated systems, which is necessary to develop reliable prediction models.

The key processes found in this thesis to dominate Cu and Cd solubility and solid-phase speciation in periodically flooded soils were determined based on the observed Cu and Cd dynamics in a weakly acidic paddy soil. In contrast to acidic soils, which become neutral under reducing conditions and return to acidic pH upon reaeration, calcareous soils maintain neutral pH irrespective of redox state (Kirk, 2004). Considering the pH-dependent adsorption behavior of trace metals in soils, and that competitive metal-sulfide precipitation may be strongly affected by metal desorption kinetics, it can be expected that trace metal dynamics in calcareous soils may differ from non-calcareous soils. For example, Khaokaew et al. (2011) reported for a highly Cd contaminated alkaline paddy soil based on X-ray absorption spectroscopic results that Cd-carbonates were a major Cd species under oxic and anoxic conditions. Small amounts of CdS were found only after extended periods of soil flooding (30−150 days). The formation of Cd and other trace metal carbonate phases in multi-metal contaminated alkaline wetland soils may significantly alter their competitive interaction during reduction and reoxidation and needs to be studied in more detail to assess the sink and source potential of alkaline vs. acidic to neutral wetland soils.
In addition to these more field related studies, there is also the need for basic research using model systems to investigate the interactions between trace metals competing for biogenic sulfide under reducing conditions, as well as their interactions with natural organic matter at various redox states. Previous studies reported the formation of Cd-containing Cu-rich metal-sulfide nanoparticles in the pore water of a contaminated floodplain soil during soil reduction (Weber et al., 2009; Hofacker et al., 2013). The EXAFS results presented in this thesis showed that at least a fraction of the Cd was present in a pure Cd-sulfide phase. Associations of Cu and Cd in a mixed-metal sulfide may therefore rather occur due to aggregation of extremely small but chemically pure Cu$_x$S and CdS crystallites. Accordingly, Tsamouras et al. (1999a) showed that co-precipitation of Cu and Cd with sulfide from supersaturated acidic solutions (pH 2.5) lead to the formation of separate CdS and CuS phases rather than to a mixed phase. In contrast, for Cu-Ni and Cu-Zn sulfides they reported the formation of solid phases with intermediate composition (Cu$_x$Zn$_{1-x}$S, Cu$_x$Ni$_{1-x}$S) (Tsamouras et al., 1998, 1999b). They attributed this different behavior to the larger difference in the atomic radius between Cu and Cd, compared to Ni and Zn. Further model studies are needed to investigate the formation and characteristics of mixed-metal sulfides of environmentally important contaminants (e.g., Cu, Cd, Pb, Zn, Ni) under natural conditions, i.e., at more natural pH conditions and in the presence of inorganic and organic ligands. The results are expected to strongly contribute to the understanding of competitive metal-sulfide formation in multi-metal contaminated soils. A further task is also the investigation of the stability of mixed-metal sulfide phases under oxic conditions. In recent studies it was reported that the oxidative release of Cd from pure CdS phases was enhanced compared to the Cd release from Cd-Zn sulfide and Cd-Fe-Zn sulfide phases (Barrett and McBride, 2007; de Livera et al., 2011), which suggested that mixed-metal sulfides can play an important role for the remobilization of trace metals during soil oxidation.

A further research challenge remained also regarding the investigation of Cu interactions and binding by NOM under strongly reducing conditions in terrestrial environments. Although the possibility of Cu complexation by reduced organic sulfur groups has been considered in previous synchrotron-based studies on Cu binding to natural organic matter (Karlsson et al., 2006; Manceau and Matynia, 2010), these studies concluded that associations of Cu with organic S are
of minor importance. However, these studies were focused on the binding of Cu(II), using humic materials that were exposed to O$_2$ during extraction, Cu sorption or measurements. Parts of the experiments presented in chapter 4 were conducted with electrochemically reduced humic acid, and anoxic conditions were maintained throughout the experiment. The spectroscopic results for the interaction of Cu with reduced humic acid in this model experiment strongly suggested the formation of Cu(I) complexes via at least one nitrogen and/or sulfur ligand group. However, the spectroscopic results could not completely unravel the coordination environment of these Cu(I)-HA complexes, due to experimental limitations, i.e., the presence of high chloride concentrations in the background electrolyte. Therefore, further research is needed to determine the coordination chemistry of Cu(I) in natural organic matter in more detail and to investigate the quantitative importance of Cu(I)-NOM complexes in redox influenced soils with various organic matter contents.
5.1. References


A. **Supporting Information to Chapter 2**

This chapter has been accepted with minor modifications for publication in *Geochimica et Cosmochimica Acta* as Supporting Information to: Fulda B., Voegelin A., Ehlert K., Kretzschmar R.. Redox transformation, solid phase speciation and solution dynamics of copper during soil reduction and reoxidation as affected by sulfate availability.
## A.1. Cu reference compounds and their evaluation for EXAFS LCF

### Table A.1. Structural information, source and measurement conditions of Cu reference compounds used in the Cu K-edge XAS study.

<table>
<thead>
<tr>
<th>Reference name</th>
<th>Source (spectra/materials) Measurement information</th>
<th>Structural information from literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu metal</td>
<td>Goodfellow 106-905-90 INE beamline; 77K</td>
<td>Coordination: 12Cu, Distance (Å): 2.54, Reference: Wyckoff (1963)</td>
</tr>
<tr>
<td>Cu(II)-clay a</td>
<td>synthesized (this study) INE beamline; 77K</td>
<td>Coordination: 40, Distance (Å): 1.95, Reference: Strawn et al. (2004)</td>
</tr>
<tr>
<td>Cu(II)-malate</td>
<td>synthesized (this study) XAS beamline; 15 K</td>
<td>Coordination: 40, Distance (Å): 1.91–1.95, Reference: Zhang (2007)</td>
</tr>
<tr>
<td>Cu(II)-humic b</td>
<td>synthesized (this study) INE beamline; 77K</td>
<td>Coordination: 40/N, Distance (Å): 1.92–1.95, Reference: Karlsson et al. (2006)</td>
</tr>
<tr>
<td>CuS prim</td>
<td>spectrum from Patrignani et al. (1997); 77K</td>
<td>Coordination: 3S, Distance (Å): 2.28, Reference: Patrignani et al. (1997)</td>
</tr>
<tr>
<td>CuS</td>
<td>Sigma Aldrich 510653 INE beamline; 77K</td>
<td>Coordination: 1.5S, Distance (Å): 2.80, Reference: Patrignani et al. (1997)</td>
</tr>
<tr>
<td>CuS (cuvellite)</td>
<td>Alfa Aesar 042925 INE beamline; 77K</td>
<td>Coordination: 2.92S, Distance (Å): 2.30, Reference: Patrignani et al. (1997)</td>
</tr>
<tr>
<td>CuS (cuvellite)</td>
<td>Alfa Aesar 042925 INE beamline; 77K</td>
<td>Coordination: 3.67S, Distance (Å): 2.27, Reference: Patrignani et al. (1997)</td>
</tr>
<tr>
<td>Cu(II)-carbamat c</td>
<td>synthesized (this study) XAS beamline; 15 K</td>
<td>Coordination: 3S, Distance (Å): 2.27, Reference: shell fit (this study)</td>
</tr>
<tr>
<td>Cu(II)-GSH d</td>
<td>synthesized (this study) INE beamline; 77K</td>
<td>Coordination: 3S, Distance (Å): 2.26, Reference: Pogor et al. (2008)</td>
</tr>
<tr>
<td>Cu(II)-S-trigonal</td>
<td>spectrum from Pufahl et al. (1997); 10K</td>
<td>Coordination: 2S, Distance (Å): 2.27, Reference: Coucoubianis et al. (1980)</td>
</tr>
<tr>
<td>Cu(II)-COP C</td>
<td>spectrum from Arnesano et al. (2003); 20 K</td>
<td>Coordination: 2-3S (Met), Distance (Å): 2.30–2.31, Reference: Arnesano et al. (2003)</td>
</tr>
</tbody>
</table>

a. **Cu(II)-clay**: 0.5 g of montmorillonite (SWy2) were equilibrated with 25 mL of a 1.57 mM Cu solution (Cu(NO₃)₂ dissolved in 50 mM MES buffer (pH 6.1) (Cu(II) loading: ~5000 mg kg⁻¹). After reaction for 24 h at room temperature under continuous shaking, the suspension was centrifuged (30 min at 3566 g) and the clay was frozen in liquid N₂ and freeze-dried.
A.1 Cu reference compounds and their evaluation for EXAFS LCF

\[ \text{Cu(II)-humic:} \quad 20 \text{ mL of a soil humic acid solution (2.27 g L}^{-1} \text{ TOC, 10 mM NaNO}_3, \text{ pH 6) were spiked with 800 } \mu\text{L of a 50 mM CuCl}_2 \text{ solution (Cu(II) loading: } \approx 55000 \text{ mg kg}^{-1} \text{), shaken slowly for 1 h, frozen in liquid N}_2 \text{ and freeze-dried.} \]

\[ \text{Cu(II)-malate:} \quad 44 \text{ mg of Cu(CO}_3\text{Cu(OH)}_2 \text{ were dissolved in 40 mL of a 100 mM L-malic acid solution (molar malate to Cu(II) ratio: 10:1) and adjusted with NaOH to pH 7. The solution was frozen in liquid N}_2 \text{ and freeze-dried.} \]

\[ \text{Cu(I)-carbamate:} \quad 20 \text{ mL of a 20 mM CuCl solution (0.1 M NaCl, 1 M HCl) was added slowly to 20 mL of a 200 mM sodium diethyldithiocarbamate trihydrate ((C}_2\text{H}_5\text{NCSSNa }3\text{H}_2\text{O}) \text{ solution (molar carbamate to Cu(I) ratio: 100:1) and adjusted with NaOH to pH 7. The black precipitate was centrifuged (30 min at 3566 g) and the residuum frozen in liquid N}_2 \text{ and freeze-dried in the glovebox vacuum chamber under anoxic conditions.} \]

\[ \text{Cu(I)-GSH:} \quad 10 \text{ mg of CuCl were diluted in 10 mL of a 0.1 M solution of L-Glutathione reduced (molar GSH to Cu(I) ration 10:1) and adjusted with NaOH solution to pH 7. Solution was shaken slowly for 2 h, shock-frozen in liquid N}_2 \text{ and freeze-dried in the glovebox vacuum chamber under anoxic conditions.} \]

**Evaluation of Cu reference spectra for EXAFS LCF by PCA-TT**

To obtain an indication for the minimum number of references needed for LCF analysis, the number of spectral components required to reproduce the sample data set was determined by principal component analysis (PCA) based on the empirical indicator (IND) function (Malinowski, 1977). To assess the suitability of reference spectra for LCF, all spectra listed in Table A.1 were evaluated by target transform testing (TT). TT was performed based on the number of significant components derived from PCA and assessed by the empirical SPOIL value (Malinowski, 1978). PCA and TT were carried out using the program SIXPack (Webb, 2005).

The principal component analysis (PCA) revealed that at least three independent spectral components are required to satisfactorily reproduce the 9 soil spectra, as indicated by a minimum in the empirical IND values (Table A.2) (Malinowski, 1977). The first three components explained 75% of the spectral variance and reproduced most sample spectra with a normalized sum of squared residuals (NSSR) < 10% (Figure A.1a). Using the first three PCA
components, potential reference spectra for LCF were evaluated by target transformation (TT)
testing, which returns an empirical SPOIL value for each reference (Figure A.1b). References
with SPOIL values <1.5 are considered excellent, 1.5–3 good, 3–4.5 fair, 4.5–6 poor and >6
unacceptable (Malinowski, 1978). Metallic copper Cu(0) had by far the lowest SPOIL (0.8) and
was therefore considered an important reference for LCF. Among the Cu(II)-O coordinated
references, Cu(II)-clay and Cu(II) organically complexed by O/N (Cu(II)-humic, Cu(II)-malate)
were all found to be excellent or good references (SPOIL 1.1–1.6), reflecting the structural
similarity of their first-shell coordination (distorted O/N octahedron) (Strawn et al., 2004;
Manceau and Matynia, 2010). All inorganic and organic Cu-S references were classified as
excellent or good (except covellite, CuS). Among these references, primitive Cu sulfide (Cu,Sprim)
had the lowest SPOIL (1.3). The poorer classification of covellite (CuS; SPOIL 3.4) compared to
other Cu-S references could be attributed to its well-developed Cu-Cu second shell, which was
absent in the soil spectra (Figure 2.4d and Figure A.2).

Table A.2. Results from principal component analysis (PCA) of 9 k^3-weighted sample EXAFS spectra (k-range = 2.5–10 Å⁻¹).

<table>
<thead>
<tr>
<th>Comp</th>
<th>Eigenvalue</th>
<th>Cum.Var.*</th>
<th>INDb</th>
<th>NSSRc (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>104.1</td>
<td>0.426</td>
<td>0.344</td>
<td>26.30</td>
</tr>
<tr>
<td>2</td>
<td>45.5</td>
<td>0.612</td>
<td>0.327</td>
<td>12.20</td>
</tr>
<tr>
<td>3</td>
<td>34.2</td>
<td>0.752</td>
<td>0.283</td>
<td>4.24</td>
</tr>
<tr>
<td>4</td>
<td>12.0</td>
<td>0.001</td>
<td>0.392</td>
<td>3.26</td>
</tr>
<tr>
<td>5</td>
<td>11.5</td>
<td>0.848</td>
<td>0.583</td>
<td>2.36</td>
</tr>
<tr>
<td>6</td>
<td>10.6</td>
<td>0.892</td>
<td>0.986</td>
<td>1.61</td>
</tr>
</tbody>
</table>

*a Cumulative variance. b Indicator function. c Normalized sum of squared residuals Σ_{spectra} Σ_i (k^3χ_{exp} - k^3χ_{recon})^2 / Σ_{spectra} Σ_i (k^3χ_{exp})^2
Figure A.1. a) $k^3$-weighted Cu K-edge EXAFS sample spectra and their reconstruction with linear combinations of the first three principal components (PCA results in Table A.2). b) EXAFS spectra of selected reference compounds and their respective target transforms calculated with the first three components from the principal component analysis. NSSR is the normalized sum of squared residuals = $\sum_i (k^3\chi_{exp} - k^3\chi_{reconst})^2 / \sum_i (k^3\chi_{exp})^2$
Figure A.2. Cu $K$-edge EXAFS spectra of various Cu-S reference compounds. a) $k^2$-weighted EXAFS spectra; b) magnitude and c) real part of the Fourier-transformed EXAFS spectra ($k$-range 2.5−10 Å$^{-1}$, dk=2.5); d) back transform ($R+\Delta R$-range = 1.2−2.2 Å, $k$-weight = 3).
A.2. Solution composition during soil reduction and reoxidation

Figure A.3. Solution dynamics during reduction and reoxidation phase for Cu-spiked (left panels) and control series (right panel). Error bars indicate the standard deviation of the experimental triplicates. The horizontal dashed line in panel c) marks roughly the average concentration of unspecified S ($S_{\text{unspec}}$) during the reduction phase in all series (calculated as the difference between total dissolved S (ICP-OES) and sulfate (IC)).
Figure A.4. a) Dissolved inorganic carbon (DIC) dynamics and b) total CO$_2$ production (TIC) calculated on soil dry-weight basis during reduction and reoxidation phase for Cu-spiked (left panels) and control series (right panel). Error bars indicate the standard deviation of the experimental triplicates.

DIC values during the reduction period were recalculated from the CO$_2$ partial pressures measured in the headspace of the incubation vials by gas chromatography (GC), using Henry’s law ($K_H = \frac{[H_2CO_3^*]}{p_{CO_2}} = 0.0345 \text{ mol L}^{-1} \text{ bar}^{-1}$ (Sander, 1999)) and DIC equilibrium constants ($K_1 = [H^+][H_2CO_3]/[H_2CO_3^+] = 10^{-6.35}$ and $K_2 = [H^+][CO_3^{2-}]/[HCO_3^-] = 10^{-10.33}$ (Stumm and Morgan, 1996)) ($\text{DIC} = H_2CO_3^* + H_2CO_3^- + CO_3^{2-}$). Total CO$_2$ production was calculated as the sum of CO$_2$ in the headspace and DIC concentrations on soil dry-weight basis. For the measurement of DIC concentrations during the reoxidation period 0.5 mL of the filtered sample solutions were placed in 20 mL crimp vials and sealed with a butyl rubber septum. After addition of 50 μL of 4M HCl solution, CO$_2$ concentrations were measured in the headspace by GC. DIC concentrations were recalculated using CO$_2$ headspace concentration, the volumes of the headspace and water phase and Henry’s law.
A.3 Sulfate solution dynamics and organic S mineralization

Figure A.5. Relation between a) sulfate reduction rate and b) sulfate release rate and total initial available sulfate amounts (sulfate\text{start}). For LSC and LS total initial sulfate was calculated from the maximal dissolved sulfate amounts at day 1 and day 10 of reduction, respectively.

In the metal-spiked series, sulfate release rates during reoxidation, as calculated for the period of linear increase in dissolved sulfate concentrations with time (LSC, MSC: 0–2 d REOX; MS: 0–7 d REOX; HS: 0–14 d REOX), perfectly correlated with the initial extractable sulfate amounts (Figure A.5b). This suggested that sulfate release rates were related to sulfide oxidation and depending on the amount of sulfide present at the end of the reduction phase. However, the sulfate release rates for the two control series deviated significantly from this correlation, and were four to five times higher than in the corresponding metal-spiked series. These higher release rates could not solely be related to formation of additional sulfide by organic S mineralization during reduction. To match the correlation of the metal spiked series 3–9 mmol kg\textsuperscript{-1} of sulfate in the control series must have been mineralized during the reduction period. These amounts were rather unrealistic, considering the by Zhou et al. (2005) reported S mineralization rates for a flooded paddy soil (0.3 mmol kg\textsuperscript{-1} within 40 days). Therefore, the higher sulfate release rates in the control series suggested that S mineralization occurred mainly during reoxidation and that the metal spike retarded S mineralization.
Figure A.6. Organic S mineralization evaluated by the increase in total inorganic sulfur (TIS) (visualized by the black solid line). $\Delta S_{\text{unspec}}$ = additional amount of unspecified S during reoxidation compared to values at day 40 of reduction (assumed to be predominantly elemental S and thiosulfate). CRS values were only available for $t = 0, 10, 40, 54$ and $68$ d. $\text{CRS}_{\text{tot}}$ for LSC-14d REOX was estimated to be at most the $\text{CRS}_{\text{tot}}$ amount in LSC-28d REOX. Sulfate and $\Delta S_{\text{unspec}}$ in the MSC series and at time points without available $\text{CRS}_{\text{tot}}$ data were plotted for comparison (b.d. = sulfate below detection limit).

The dynamics in total inorganic sulfur (TIS) (Figure A.6) were in line with the observed sulfate release rates. TIS was calculated as the sum of $\text{CRS}_{\text{tot}}$ and the amounts of sulfate and unspecified S in solution. As discussed in section 2.3.1, we assumed that the unspecified S at the end of the reduction period was mainly S in DOM and that the increase in unspecified S during reoxidation, especially in the MSC and HS series, reflected formation of thiosulfate and elemental S as intermediates during the oxidation of sulfide to sulfate (Burton et al., 2006). Therefore, the formation of thiosulfate and elemental S during reoxidation was estimated from the difference between unspecified S concentrations after 40 days of reduction, and the values during reoxidation (Figure A.3c). We observed an increase in TIS contents over the whole incubation
cycle in all series (0.7–0.8 mmol kg\(^{-1}\) in LS, MS, and HS; 1.4 mmol kg\(^{-1}\) in LSC), which pointed to mineralization of organic S. As already inferred from the sulfate release rates, mineralization of organic S mostly occurred during the reoxidation period in the metal spiked series and was more intense in the control series.

### A.4. Cu solid phase partitioning analyzed by sequential extraction

**Table A.3.** Results from the sequential extraction of Cu. Errors mean ± standard deviation of triplicates. Estimated values for missing data in the MS series (description see below) are marked in italic numbers.

<table>
<thead>
<tr>
<th></th>
<th>F1 (%)</th>
<th>F2 (%)</th>
<th>F3 (%)</th>
<th>F4 (%)</th>
<th>F5 (%)</th>
<th>RES(^a) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>low sulfate LS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2d EQ</td>
<td>0.9 ±0.0</td>
<td>50 ±1.8</td>
<td>35 ±0.3</td>
<td>2.8 ±0.1</td>
<td>2.7 ±0.2</td>
<td>9.0</td>
</tr>
<tr>
<td>10d RED</td>
<td>4.5 ±0.1</td>
<td>21 ±0.4</td>
<td>39 ±0.3</td>
<td>4.7 ±0.3</td>
<td>12 ±0.4</td>
<td>19</td>
</tr>
<tr>
<td>40d RED</td>
<td>0.0 ±0.0</td>
<td>0.5 ±0.6</td>
<td>19 ±2.0</td>
<td>15 ±1.2</td>
<td>38 ±1.9</td>
<td>27</td>
</tr>
<tr>
<td>28d REOX</td>
<td>0.3 ±0.0</td>
<td>39 ±0.9</td>
<td>33 ±0.7</td>
<td>3.4 ±0.0</td>
<td>9.0 ±0.2</td>
<td>15</td>
</tr>
<tr>
<td><strong>medium sulfate MS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2d EQ</td>
<td>0.9 ±0.0</td>
<td>49 ±0.9</td>
<td>35 ±0.3</td>
<td>2.5 ±0.1</td>
<td>6.2 ±0.3</td>
<td>5.9</td>
</tr>
<tr>
<td>10d RED</td>
<td>1.4 ±0.1</td>
<td>17 ±0.6</td>
<td>34 ±1.3</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>40d RED</td>
<td>0.0 ±0.0</td>
<td>0.0 ±0.0</td>
<td>0.3 --</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>28d REOX</td>
<td>0.5 ±0.0</td>
<td>33 ±0.7</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>high sulfate HS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2d EQ</td>
<td>0.8 ±0.0</td>
<td>48 ±0.4</td>
<td>36 ±0.3</td>
<td>2.2 ±0.1</td>
<td>10 ±0.3</td>
<td>3.2</td>
</tr>
<tr>
<td>10d RED</td>
<td>3.8 ±0.4</td>
<td>15 ±0.2</td>
<td>41 ±0.6</td>
<td>4.0 ±0.2</td>
<td>30 ±3.6</td>
<td>6.1</td>
</tr>
<tr>
<td>40d RED</td>
<td>0.0 ±0.0</td>
<td>0.0 ±0.0</td>
<td>0.1 ±0.1</td>
<td>5.0 ±0.1</td>
<td>78 ±4.2</td>
<td>17</td>
</tr>
<tr>
<td>28d REOX</td>
<td>0.3 ±0.0</td>
<td>21 ±1.3</td>
<td>28 ±1.1</td>
<td>1.9 ±0.6</td>
<td>40 ±1.1</td>
<td>9.1</td>
</tr>
</tbody>
</table>

\(^a\) Residual fraction, calculated as the difference between total Cu concentrations (XRF) and the sum of all fractions.

Table A.3 shows Cu partitioning in the soil according to the five-step sequential extraction procedure. The samples of the medium sulfate series were only extracted until step 2. For the comparison of the sequential extraction data with the results derived by EXAFS LCF (Figure 2.5), the missing values in the MS series (labeled as 0, 2, 5 in Figure 2.5) were estimated to achieve a reasonable number of data points in the correlations. It can be assumed that Cu speciation after the 2-day equilibration period was the same for all series. Fractions F3–F5 for the MS-2d EQ
sample (label 0) were therefore calculated as the average of the fractions in the LS- and HS-2d EQ samples. For the MS-10d RED sample (label 2) fraction F3 was approximated under the assumption that the ratio between F3 and F1+F2 was constant for all samples at this time point. F3 was therefore calculated using the measured sum of F1 and F2 and the average F3/(F1+F2) ratio of LS- and HS-10d RED (1.84). The sum of F4+F5+RES was than calculated as the difference to 100 %. For the MS-40d RED sample (label 5) data from a preliminary test were available which showed that only 0.3 % of total Cu was extracted into fraction F3. The sum of F4+F5+RES was therefore set to 100 %.

A.5. **Comparison of soil EXAFS spectra measured under different conditions**

For the samples of the MS and HS series, preliminary Cu XAS measurements were carried out at the ANKA XAS beamline at room temperature (Figure A.7). The results from LCF analysis of these spectra are listed in Table A.4. In Figure A.8, LCF results for spectra recorded at room temperature (XAS beamline) are compared to results from spectra recorded at 77 K (INE beamline). For metallic Cu, LCF results from both measurements were in close agreement where fractions were well-quantifiable (Figure A.8c). With respect to the fractions of Cu(II)-clay and Cu$_{x}$S$_{prim}$, systematic differences between LCF results for RT-spectra and 77-K-spectra were observed (Figures A.8a and b). The fractions of Cu(II)-clay derived from spectra measured at 77 K were about 27% (relative) lower than fractions derived from room-temperature spectra recorded at XAS, whereas the respective Cu$_{x}$S$_{prim}$ fractions were on average 17 % (absolute) higher. These deviations suggested a more reduced oxidation state of Cu in samples recorded at the INE beamline at 77 K than in samples recorded at the XAS beamline at room temperature. Although, sample cooling was expected to slow down radiation-induced sample changes, the differences between the two spectra sets could possibly be due to beam-induced Cu reduction (Strawn and Baker, 2009; Manceau and Matynia, 2010) at the INE beamline, where the use of a focused beam resulted in a 25-30 times higher flux density than at the XAS beamline. However, the Cu-sulfide fractions derived from the room-temperature spectra may also have been underestimated because the reference spectrum of Cu$_{x}$S$_{prim}$ was recorded at 77 K (Pattrick et al.,
1997) and likely exhibited a higher signal amplitude than amorphous CuS recorded at room temperature.

In conclusion, these considerations suggest that Cu-sulfide fractions obtained by LCF analysis of spectra recorded at the INE beamline at 77 K represent the upper limit for the fraction of S-coordinated Cu(I), whereas the Cu-sulfide fractions derived from the room-temperature spectra recorded at the XAS beamline represent the lower limit. With respect to the balance between sulfide formation derived from CRS analyses and Cu-S coordination inferred from XAS analysis, the results reported in Table 2.4 and Table A.4 suggest that at least in the medium-sulfate and low-sulfate series and at least temporarily, Cu-S coordination exceeded the extent of Cu-S coordination expected from sulfide formation, which can be taken as an indication for potential Cu(I) coordination by organic S groups.
Figure A.7. a) Comparison of $k^2$-weighted EXAFS sample spectra measured at room temperature (RT, XAS beamline) and 77 K (INE beamline). b) LCF analysis of RT sample spectra over the $k$-range 2.5 to 8 Å$^{-1}$ using Cu(II)-clay (RT), Cu$\text{Sprim}$ (77 K) and Cu(0) (RT) as reference spectra. LCF results are given in Table A.4.
Table A.4. LCF analysis of $k^2$-weighted EXAFS spectra measured at room temperature at XAS beamline (Figure A.7b) over a $k$-range of 2.5 to 8 Å$^{-1}$. Individual fitted fractions were constrained to range from 0 to 1, the sum of all fractions was not constrained.

<table>
<thead>
<tr>
<th>incubation Series</th>
<th>Cu(II)-clay (%)</th>
<th>Cu metal (%)</th>
<th>Cu$_{Spri}$ (%)</th>
<th>Sum (%)</th>
<th>NSSR $^a$ (%)</th>
<th>$\Delta$ Sulfide $^b$ (mmol/kg) $x = 1$</th>
<th>$x = 1.39$</th>
</tr>
</thead>
<tbody>
<tr>
<td>medium sulfate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2d EQ</td>
<td>96</td>
<td>--</td>
<td>1.6</td>
<td>97</td>
<td>3.8</td>
<td>$+0.4 \pm 0.1$</td>
<td>$+0.4 \pm 0.1$</td>
</tr>
<tr>
<td>10d RED</td>
<td>45</td>
<td>17</td>
<td>19</td>
<td>80</td>
<td>7.7</td>
<td>$-0.5 \pm 0.2$</td>
<td>$-0.3 \pm 0.2$</td>
</tr>
<tr>
<td>40d RED</td>
<td>20</td>
<td>0.6</td>
<td>74</td>
<td>94</td>
<td>5.8</td>
<td>$-0.5 \pm 0.3$</td>
<td>$+0.4 \pm 0.3$</td>
</tr>
<tr>
<td>14d REOX</td>
<td>64</td>
<td>--</td>
<td>28</td>
<td>91</td>
<td>3.7</td>
<td>$+0.3 \pm 0.3$</td>
<td>$+0.7 \pm 0.3$</td>
</tr>
<tr>
<td>high sulfate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10d RED</td>
<td>44</td>
<td>6.7</td>
<td>23</td>
<td>74</td>
<td>15</td>
<td>$-0.2 \pm 0.2$</td>
<td>$+0.1 \pm 0.3$</td>
</tr>
<tr>
<td>40d RED</td>
<td>21</td>
<td>--</td>
<td>85</td>
<td>106</td>
<td>4.3</td>
<td>$+2.3 \pm 0.6$</td>
<td>$+3.4 \pm 0.6$</td>
</tr>
<tr>
<td>low sulfate - recalculated $^c$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10d RED</td>
<td>48</td>
<td>13</td>
<td>6.5</td>
<td></td>
<td></td>
<td>$+0.1 \pm 0.2$</td>
<td>$+0.2 \pm 0.2$</td>
</tr>
<tr>
<td>40d RED</td>
<td>23</td>
<td>36</td>
<td>35</td>
<td></td>
<td></td>
<td>$-1.0 \pm 0.2$</td>
<td>$-0.6 \pm 0.2$</td>
</tr>
<tr>
<td>14d REOX</td>
<td>80</td>
<td>--</td>
<td>11</td>
<td></td>
<td></td>
<td>$+0.0 \pm 0.2$</td>
<td>$+0.1 \pm 0.2$</td>
</tr>
</tbody>
</table>

$^a$ Normalized sum squared residuals = $\sum (k^2\chi_{exp} - k^2\chi_{fit})^2 / \sum (k^2\chi_{exp})^2$. $^b$ Difference between the maximal amount of sulfide (CRS$_{max}$) and the fitted sulfide amount (LCF) calculated under the assumption of Cu$S$ stoichiometry of $x = 1$ (i.e., covellite) or $x = 1.39$ (i.e., spionkopite), with uncertainties from Gaussian error propagation. Negative values indicate that the amount of sulfide derived from LCF exceeds the CRS$_{max}$ amount in the sample. $^c$ Samples from the LS series were only measured at 77 K. The tabulated values were calculated from the LCF results for spectra recorded at 77 K (Table 2.4) using the linear regressions shown in Figure A.8.

Figure A.8. Comparison of three-component LCF results ($k = 2.5$–8 Å$^{-1}$) for EXAFS spectra recorded at room temperature at XAS beamline and at 77 K at INE beamline. a) Cu(II)-clay, b) Cu$_{Spri}$ and c) metallic Cu.
Appendix A

A.6. EXAFS LCF results of the four-component fits with Cu(I)-S$_{org}$ references

Figure A.9. Results from the EXAFS LCF with a) three components (Cu$_{S_{prim}}$ free) compared to the four-component fits (Cu$_{S_{prim}}$ fix to CRS$_{max}$) with b) Cu(I)-carbamate, c) Cu(I)-GSH, d) Cu(I)-trigonal and e) Cu(I)-CopC. Values are reported in Table A.5.
Table A.5. Results from the individual LCF with Cu$_{S_{\text{prim}}}$ fixed based on CRS$_{\text{max}}$ (assuming a Cu:S ratio of x=1 in Cu$_x$S) and one of the four different Cu(I)-S$_{\text{org}}$ reference spectra. The average and standard deviation from the four LCF are reported in Table 2.5.

<table>
<thead>
<tr>
<th></th>
<th>Cu(I)-Carb % (sum/NSSR)</th>
<th>Cu(I)-GSH % (sum/NSSR)</th>
<th>Cu(I)-S-trig % (sum/NSSR)</th>
<th>Cu(I)-CopC % (sum/NSSR)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>low sulfate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10d RED</td>
<td>11 (65 / 0.12)</td>
<td>11 (65 / 0.12)</td>
<td>21 (73 / 0.12)</td>
<td>19 (72 / 0.12)</td>
</tr>
<tr>
<td>40d RED</td>
<td>26 (86 / 0.02)</td>
<td>21 (81 / 0.03)</td>
<td>37 (93 / 0.03)</td>
<td>38 (96 / 0.02)</td>
</tr>
<tr>
<td>14d REOX</td>
<td>14 (82 / 0.22)</td>
<td>17 (84 / 0.21)</td>
<td>25 (89 / 0.23)</td>
<td>14 (82 / 0.25)</td>
</tr>
<tr>
<td><strong>medium sulfate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2d EQ</td>
<td>0.4 (84 / 0.14)</td>
<td>-- (83 / 0.14)</td>
<td>-- (83 / 0.14)</td>
<td>-- (83 / 0.14)</td>
</tr>
<tr>
<td>10d RED</td>
<td>21 (72 / 0.06)</td>
<td>18 (69 / 0.07)</td>
<td>32 (80 / 0.07)</td>
<td>30 (80 / 0.06)</td>
</tr>
<tr>
<td>40d RED</td>
<td>16 (93 / 0.20)</td>
<td>26 (98 / 0.17)</td>
<td>39 (108 / 0.19)</td>
<td>25 (99 / 0.21)</td>
</tr>
<tr>
<td>14d REOX</td>
<td>19 (90 / 0.17)</td>
<td>20 (92 / 0.16)</td>
<td>32 (100 / 0.18)</td>
<td>19 (91 / 0.21)</td>
</tr>
<tr>
<td><strong>high sulfate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10d RED</td>
<td>15 (72 / 0.20)</td>
<td>12 (69 / 0.22)</td>
<td>17 (73 / 0.23)</td>
<td>17 (74 / 0.22)</td>
</tr>
<tr>
<td>40d RED</td>
<td>10 (115 / 0.14)</td>
<td>10 (115 / 0.15)</td>
<td>18 (121 / 0.15)</td>
<td>13 (118 / 0.15)</td>
</tr>
</tbody>
</table>

NSSR is the normalized sum of squared residuals = $\sum_i (k^3\chi_{\text{exp}} - k^3\chi_{\text{fit}})^2 / \sum_i (k^3\chi_{\text{exp}})^2$
A.7. Formation of mixed Cu-Fe sulfide mineral phases

Figure A.10. Cu sulfide references in comparison to HS-40d RED. a) $k^3$-weighted EXAFS spectra; b) magnitude and real part of the Fourier-transformed EXAFS spectra ($k$-range 2.5–10 Å$^{-1}$, $dk=2.5$); c) and d) back transform over the first shell and second shell of the Fourier transformed EXAFS spectra ($k$-weight = 3).
A.8. References


Appendix A


B. Supporting Information to Chapter 3

This chapter has been submitted with minor modifications for publication in Environmental Science and Technology as Supporting Information to: Fulda B., Voegelin A., Kretzschmar R.. Redox-controlled changes in cadmium solubility and solid-phase speciation in a paddy soil as affected by reducible sulfate and copper.
B.1. Source or synthesis and structural characterization of Cd references

Source or synthesis of Cd reference compounds

\textbf{CdCO}_3: Purchased form Sigma Aldrich (229504).

\textbf{Cd(NO}_3\textbf{)}_2: 200 mM Cd(NO}_3\textbf{)}_2 solution. Solution was frozen for XAS measurement.

\textbf{Cd-clay}: 0.5 g of montmorillonite (SWy2) were equilibrated with 25 mL of a 0.89 mM Cd(NO}_3\textbf{)}_2 dissolved in 50 mM MES buffer (pH 6.1), leading to a Cd loading of \(~5000 \text{ mg kg}^{-1}\). After reaction for 24 h at room temperature under continuous shaking, the suspension was centrifuged (30 min at 3566 \(g\)) and the clay was frozen in liquid N\(_2\) and freeze-dried.

\textbf{Cd-goethite}: 0.5 g of goethite were equilibrated with 25 mL of a 1.42 mM Cd(NO}_3\textbf{)}_2 dissolved in 50 mM MES buffer (pH 6.1), leading to a Cd loading of \(~8000 \text{ mg kg}^{-1}\). After reaction for 24 h at room temperature under continuous shaking, the suspension was centrifuged (30 min at 3566 \(g\)) and the clay was frozen in liquid N\(_2\) and freeze-dried.

\textbf{Cd-carboxyl}: Synthesis was performed after Karlsson et al. (2005): 10g of BioRex® 70 carboxylic resin (Bio-Rad) was equilibrated for 30 min with 50 mL 1M HCl and washed ten times with 50 mL Milli-Q® water until pH was at about 6. 50 ml of a 3.29 mM Cd(NO}_3\textbf{)}_2 solution were equilibrated for 24 h with 2.5 g of the wet resin (1.85 g dry weight) leading to a loading of \(~10000 \text{ mg kg}^{-1}\). The final pH after 24 h of equilibration was pH 3. Solution was decanted and resin was then ground in a agate mortar and dried in the glovebox vacuum chamber.

\textbf{Cd-thiol (pH9 and pH3)}: Synthesis was performed after Karlsson et al. (2005): 10 g of Ambersep GT74® thiol resin (Supelco) was equilibrated for 30 min with 50 mL 1M HCl and washed ten times with 50 mL Milli-Q® water until pH was at about 6. 50 ml of a 2.45 mM Cd(NO}_3\textbf{)}_2 solution were equilibrated for 24 h with 2.5 g of the wet resin (1.375 g dry weight) leading to a loading of \(~10000 \text{ mg kg}^{-1}\). The pH value was adjusted with NaOH to pH 9 (or pH 3). Solution was decanted and resin was then ground in a agate mortar and dried in the glovebox vacuum chamber. All redox sensitive steps were performed in a glovebox (O\(_2\) < 1 ppm).
**Cd-humic:** Stock solution (5 g L⁻¹) of a purified soil humic acid (Christl et al., 2000) were diluted together with 0.5 mM Cd(NO₃)₂ and 0.5 M NaCl solution to final concentrations of 1 g L⁻¹ HA, 0.02 mM Cd(NO₃)₂ and 0.01 mM NaCl, leading to a Cd loading of ~2000 mg kg⁻¹. The pH value was adjusted with NaOH to pH 7. After reaction for 24 h at room temperature under continuous shaking, the solution was frozen in liquid N₂ and freeze-dried.

**CdS:** Purchased from Sigma Aldrich (217921). XRD analysis revealed that CdS was >90% in the hexagonal form (alpha-CdS, greenockite).

**Characterization of Cd reference compounds by shell-fitting**

Cd reference EXAFS spectra were characterized by multiple $k$-weight ($k = 1, 2, 3$) shell-fitting in $R$-space using the software code Artemis (Ravel and Newville, 2005). Theoretical EXAFS phase-shift and amplitude functions for various single scattering paths were calculated with FEFF v.8.4 (Ankudinov et al., 1998) based on the structure of CdCO₃ (Graf, 1961) and CdS (Stevenson et al., 1984). The fits and the corresponding EXAFS parameters are presented in Table B.1 and Figure B.1. Fitted distances for Cd-O, Cd-S, Cd-C and Cd-Cd single scattering paths in CdCO₃ and CdS were in agreement with values expected based on their crystallographic structure (Graf, 1961; Stevenson et al., 1984). The amplitude correction factors ($S₀²$) for CdCO₃ was high (1.33), but in line with reported elevated $S₀²$ values for some specific elements including Cd (Li et al., 1995). For the analysis of the other reference compounds, the $S₀²$ for Cd-O and for Cd-S and Cd-Cd single-scattering paths were fixed to the values derived for CdCO₃ and CdS, respectively. Energy-shift parameters ($ΔE₀$) and the Debye Waller factors ($σ²$) were fixed as indicated in Table B.1.

The fitted coordination numbers (CN) and distances ($R$) for Cd-O, Cd-S and Cd-Cd are in agreement with values reported by other authors for similar Cd compounds (Parkman et al., 1999; Frenkel et al., 2001; Karlsson et al., 2005; Vasconcelos et al., 2008). The reference compounds characterized by exclusive first-shell coordination to O – Cd(NO₃)₂, Cd-clay, Cd-goethite and Cd-carboxyl – had ~6-7 O atoms at a distance of 2.27-2.30 Å. In the Cd-humic and the Cd-thiol references, Cd is coordinated by a mixture of ~1-3 O atoms at a distance of 2.29-2.32 Å and ~2-3 S atoms at a distance of 2.52-2.58 Å. In accordance with Karlsson et al (2005)
Cd-S coordination in the Cd-thiol synthesized at pH 9 was higher than in the reference synthesized at pH 3.

<table>
<thead>
<tr>
<th>Table B.1. EXAFS parameters determined by multiple k-weight (k = 1, 2, 3) shell-fitting of Cd K-edge EXAFS reference compound spectra (k-range = 2.5-9.2 Å⁻¹, dk = 2.5) in R-space (1.1-4.5 Å). Parameters in bold were fixed to their theoretical values, parameters in italics were fixed as indicated in the footnotes.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Path</strong></td>
</tr>
<tr>
<td>CdCO₃</td>
</tr>
<tr>
<td>Cd-C</td>
</tr>
<tr>
<td>Cd-O</td>
</tr>
<tr>
<td>Cd-Cd</td>
</tr>
<tr>
<td>CdS</td>
</tr>
<tr>
<td>Cd-Cd</td>
</tr>
<tr>
<td>Cd(NO₃)₂</td>
</tr>
<tr>
<td>Cd-clay</td>
</tr>
<tr>
<td>Cd-goethite</td>
</tr>
<tr>
<td>Cd-carboxyl</td>
</tr>
<tr>
<td>Cd-humic</td>
</tr>
<tr>
<td>Cd-S</td>
</tr>
<tr>
<td>Cd-thiol (pH 3)</td>
</tr>
<tr>
<td>Cd-S</td>
</tr>
<tr>
<td>Cd-thiol (pH 9)</td>
</tr>
<tr>
<td>Cd-S</td>
</tr>
</tbody>
</table>

* fixed to S₀² value form CaCO₃; b fixed to average σ² value from Cd(NO₃)₂, Cd-clay, Cd-goethite and Cd-carboxyl; c fixed to σ² value for Cd-S path given by Karlsson et al. (2006); d fixed to S₀² value form CdS; e fixed to average ΔE₀ value from Cd(NO₃)₂, Cd-clay, Cd-goethite and Cd-carboxyl; f fixed to ΔE₀ from CdS; g Normalized sum of squared residuals = Σᵣᵢ ((dataᵢ - fit)² / Σᵢ (dataᵢ)²).
Figure B.1. (a) Normalized Cd K-edge XANES spectra, (b) $k^3$-weighted EXAFS spectra, (c) Fourier-transform magnitudes and (d) real parts of various Cd reference compounds. The Fourier transforms were calculated over a $k$-range of 2.5-9.2 Å$^{-1}$ (Kaiser-Bessel window, $dk = 2.5$). Gray lines represent experimental data and red dotted lines shell-fits. Shell-fits were performed in $R$-space (at $k$-weights 1, 2 and 3) over the $R + \Delta R$-range 1.1-4.5 Å. Shell-fit parameters are summarized in Table B.1.

Evaluation of Cd reference spectra for EXAFS LCF by PCA-TT

The minimum number of spectral components required to reproduce the data set of 17 sample EXAFS spectra (listed in Table B.5) was determined by principal component analysis (PCA) based on the empirical indicator (IND) function (Malinowski, 1977). To assess the suitability of the various Cd reference spectra for linear combination fitting (LCF), all reference spectra were evaluated by target transform testing (TT). TT was performed based on the number of significant components derived from PCA and assessed by the empirical SPOIL value (Malinowski, 1978). PCA and TT were carried out using the program SIXPack (Webb, 2005).
Principal component analysis (PCA) revealed that at least two independent spectral components were needed to satisfactorily reproduce the 17 soil spectra, as indicated by the minimum in the empirical IND value (Malinowski, 1977) (Table B.2). The first two PCA components accounted for only 55% of the total variance in the dataset, reflecting the relatively high noise level in the Cd K-edge EXAFS spectra collected on soil samples containing only 22 mg kg\(^{-1}\) Cd. Using the first two PCA components, the Cd reference spectra were evaluated by target transform (TT) testing, which returns an empirical SPOIL value for each reference. References with SPOIL values <1.5 are considered excellent, 1.5–3 good, 3–4.5 fair, 4.5–6 poor and >6 unacceptable (Malinowski, 1978). With exception of CdCO\(_3\) and Cd-humic, all other references fell in the categories excellent to fair (Table B.3). The seven references with a SPOIL < 4.5 were used for a first series of LCF analyses comparing all possible one- to three-component fits. In the fitting procedure the sum of all fitted fractions was constrained to 100%. The best fit was chosen based on the normalized sum of squared residuals (NSSR = \(\sum (k^2\chi_{\text{exp}} - k^2\chi_{\text{fit}})^2 / \sum (k^2\chi_{\text{exp}})^2\)). Starting from the best one-component fit (\(n = 1\)), the best \(n + 1\) component fit was only considered to be significantly better than the best \(n\)-component fit if its NSSR was at least 10% (relative) lower. To reduce the number of reference compounds, especially with respect to references for O-coordinated Cd species, Cd-carboxyl, Cd-thiol (pH9) and CdS as the three references most frequently included in the best LCF (Table B.3) were chosen for the final EXAFS LCF analyses presented in Figure 3.2 and Table B.5.

### Table B.2. Results from principal component analysis (PCA) of 17 \(k^3\)-weighted sample EXAFS spectra (\(k\)-range = 2.5–9.2 Å\(^{-1}\)).

<table>
<thead>
<tr>
<th>Comp</th>
<th>Eigenvalue</th>
<th>Variance</th>
<th>Cum.Var.(^a)</th>
<th>IND(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95.0</td>
<td>0.413</td>
<td>0.413</td>
<td>0.049</td>
</tr>
<tr>
<td>2</td>
<td>32.1</td>
<td>0.139</td>
<td>0.552</td>
<td>0.039</td>
</tr>
<tr>
<td>3</td>
<td>12.0</td>
<td>0.052</td>
<td>0.604</td>
<td>0.043</td>
</tr>
<tr>
<td>4</td>
<td>10.2</td>
<td>0.044</td>
<td>0.649</td>
<td>0.048</td>
</tr>
<tr>
<td>5</td>
<td>9.0</td>
<td>0.039</td>
<td>0.688</td>
<td>0.055</td>
</tr>
</tbody>
</table>

\(^a\)Cumulative variance. \(^b\)Indicator function.
Table B.3. Target testing (TT) of reference spectra using the first two components obtained by PCA (Table B.2) and the number of times each reference occurred in the best LCF of soil EXAFS spectra using all reference with a SPOIL value < 4.5. The three most frequently occurring references (marked bold italics) were used for final EXAFS LCF analyses presented in Figure 3.2 and Table B.5.

<table>
<thead>
<tr>
<th>Reference</th>
<th>SPOIL</th>
<th>NSSR (%)</th>
<th>LCF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd-clay</td>
<td>0.5</td>
<td>1.9</td>
<td>4</td>
</tr>
<tr>
<td>CdS</td>
<td>1.2</td>
<td>3.1</td>
<td>8</td>
</tr>
<tr>
<td>Cd-goethite</td>
<td>1.7</td>
<td>5.9</td>
<td>2</td>
</tr>
<tr>
<td>Cd-carboxyl</td>
<td>1.8</td>
<td>6.0</td>
<td>8</td>
</tr>
<tr>
<td>Cd-thiol (pH9)</td>
<td>2.1</td>
<td>3.6</td>
<td>16</td>
</tr>
<tr>
<td>Cd-thiol (pH3)</td>
<td>3.0</td>
<td>6.2</td>
<td>0</td>
</tr>
<tr>
<td>Cd(NO₃)₂</td>
<td>3.1</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>CdCO₃</td>
<td>5.0</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Cd-humic</td>
<td>6.4</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>
B.2. Changes in $E_h$, pH and dissolved Fe and Mn during soil reduction and reoxidation

Figure B.2. Solution dynamics during soil reduction and reoxidation. Error bars indicate the standard deviation of experimental triplicates. Data for low-, medium- and high-sulfate series with Cd and Cu spike are from Fulda et al. (2013).
Figure B.3. Rate of dissolved Cd decrease versus molar initial soil sulfate/\((Cu+Cd)\) ratio for all incubation series. Cd decrease rates were calculated for the periods over which dissolved Cd decreased nearly linearly (LC-HCu: 15-40d; MS-HCu: 10-20d, HS-HCu: 10-20d and MS-LCu: 1-5d).
B.3. Sequential extraction results

Table B.4. Two-step sequential extraction results (mean values and standard deviation (SD) of three individual extractions). Values reported as percentage of total Cd determined by XRF.

<table>
<thead>
<tr>
<th></th>
<th>Step 1: CaCl₂ mean (%)</th>
<th>SD (%)</th>
<th>Step 2: Na-Acetate mean (%)</th>
<th>SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LS-HCu</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2dEQ</td>
<td>89</td>
<td>1.2</td>
<td>25</td>
<td>1.0</td>
</tr>
<tr>
<td>10dRED</td>
<td>56</td>
<td>0.5</td>
<td>47</td>
<td>1.0</td>
</tr>
<tr>
<td>40dRED</td>
<td>23</td>
<td>1.5</td>
<td>68</td>
<td>4.7</td>
</tr>
<tr>
<td>28dREOX</td>
<td>47</td>
<td>1.0</td>
<td>54</td>
<td>1.1</td>
</tr>
<tr>
<td><strong>MS-HCu</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2dEQ</td>
<td>78</td>
<td>2.5</td>
<td>26</td>
<td>0.4</td>
</tr>
<tr>
<td>10dRED</td>
<td>48</td>
<td>1.6</td>
<td>51</td>
<td>1.2</td>
</tr>
<tr>
<td>40dRED</td>
<td>--</td>
<td>--</td>
<td>18</td>
<td>0.7</td>
</tr>
<tr>
<td>28dREOX</td>
<td>49</td>
<td>0.8</td>
<td>54</td>
<td>1.3</td>
</tr>
<tr>
<td><strong>HS-HCu</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2dEQ</td>
<td>75</td>
<td>0.8</td>
<td>25</td>
<td>0.6</td>
</tr>
<tr>
<td>10dRED</td>
<td>68</td>
<td>3.4</td>
<td>40</td>
<td>1.8</td>
</tr>
<tr>
<td>40dRED</td>
<td>--</td>
<td>--</td>
<td>4.8</td>
<td>0.2</td>
</tr>
<tr>
<td>28dREOX</td>
<td>49</td>
<td>3.7</td>
<td>54</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>MS-LCu</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2dEQ</td>
<td>64</td>
<td>1.5</td>
<td>36</td>
<td>1.2</td>
</tr>
<tr>
<td>10dRED</td>
<td>--</td>
<td>--</td>
<td>42</td>
<td>1.0</td>
</tr>
<tr>
<td>40dRED</td>
<td>--</td>
<td>--</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>28dREOX</td>
<td>39</td>
<td>1.3</td>
<td>56</td>
<td>0.7</td>
</tr>
</tbody>
</table>
B.4. Analysis of X-ray absorption spectra of soil samples

LCF analysis of Cd K-edge EXAFS spectra

Table B.5. LCF results for the $k^3$-weighted EXAFS spectra (Figure 3.2). Statistical fit uncertainties are given in brackets.

<table>
<thead>
<tr>
<th></th>
<th>Cd-carboxyl (%)</th>
<th>Cd-thiol (pH9) (%)</th>
<th>CdS (%)</th>
<th>NSSR (%) a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LS-HCu</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2d EQ</td>
<td>76 (± 4.7)</td>
<td>24 (± 7.6)</td>
<td>0 (± 9.0)</td>
<td>7.9</td>
</tr>
<tr>
<td>20d RED</td>
<td>50 (± 5.8)</td>
<td>50 (± 8.2)</td>
<td>0 (± 10.0)</td>
<td>22</td>
</tr>
<tr>
<td>40d RED</td>
<td>11 (± 4.7)</td>
<td>60 (± 6.8)</td>
<td>30 (± 8.3)</td>
<td>7.9</td>
</tr>
<tr>
<td>14d REOX</td>
<td>72 (± 6.8)</td>
<td>28 (± 9.1)</td>
<td>0 (± 11.4)</td>
<td>26</td>
</tr>
<tr>
<td>28d REOX</td>
<td>70 (± 4.7)</td>
<td>30 (± 5.6)</td>
<td>0 (± 7.3)</td>
<td>12</td>
</tr>
<tr>
<td><strong>MS-HCu</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2d EQ</td>
<td>80 (± 4.6)</td>
<td>20 (± 5.5)</td>
<td>0 (± 7.1)</td>
<td>11</td>
</tr>
<tr>
<td>20d RED</td>
<td>16 (± 5.4)</td>
<td>58 (± 7.9)</td>
<td>26 (± 9.5)</td>
<td>9.4</td>
</tr>
<tr>
<td>40d RED</td>
<td>0 (± 0.0)</td>
<td>49 (± 0.0)</td>
<td>51 (± 0.0)</td>
<td>4.9</td>
</tr>
<tr>
<td>14d REOX</td>
<td>62 (± 4.6)</td>
<td>38 (± 6.3)</td>
<td>0 (± 7.8)</td>
<td>12</td>
</tr>
<tr>
<td>28d REOX</td>
<td>66 (± 6.7)</td>
<td>34 (± 8.8)</td>
<td>0 (± 11.0)</td>
<td>26</td>
</tr>
<tr>
<td><strong>HS-HCu</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20d RED</td>
<td>1.0 (± 4.0)</td>
<td>55 (± 5.8)</td>
<td>45 (± 7.0)</td>
<td>4.4</td>
</tr>
<tr>
<td>40d RED</td>
<td>0.0 (± 0.0)</td>
<td>37 (± 0.0)</td>
<td>64 (± 0.0)</td>
<td>6.4</td>
</tr>
<tr>
<td><strong>MS-LCu</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2d EQ</td>
<td>73 (± 4.4)</td>
<td>27 (± 5.5)</td>
<td>0 (± 7.0)</td>
<td>12</td>
</tr>
<tr>
<td>5d RED</td>
<td>36 (± 5.5)</td>
<td>27 (± 8.0)</td>
<td>37 (± 9.7)</td>
<td>10</td>
</tr>
<tr>
<td>40d RED</td>
<td>0.0 (± 0.0)</td>
<td>31 (± 0.0)</td>
<td>69 (± 0.0)</td>
<td>6.8</td>
</tr>
<tr>
<td>14d REOX</td>
<td>58 (± 5.5)</td>
<td>42 (± 6.5)</td>
<td>0 (± 8.5)</td>
<td>20</td>
</tr>
<tr>
<td>28d REOX</td>
<td>61 (± 4.4)</td>
<td>39 (± 5.5)</td>
<td>0 (± 7.1)</td>
<td>12</td>
</tr>
</tbody>
</table>

a Normalized sum of squared residuals $= \sum (k^3\chi_{exp} - k^3\chi_{th})^2 / \sum (k^3\chi_{exp})^2$

Shell-fitting analysis of Cd K-edge EXAFS spectra

In addition to EXAFS LCF analysis, shell-fitting was used to characterize Cd coordination in the soil samples. Shell-fits at multiple $k$-weights (1, 2, 3) were run in $R$-space ($k$-range 2.5-9.2 Å⁻¹; $R + \Delta R$-range 1.1-4.5 Å) using first-shell Cd-O and Cd-S single-scattering paths as well as a second-shell Cd-Cd path representative for second-shell Cd in crystalline CdS. Based on these three
paths, we compared three fitting models including one, two or three paths ($S_0^2$, $\Delta E_0$ and $\sigma^2$ were fixed to values derived from shell-fits of Cd reference compounds as described in section B.1):

**Model 1a** (1 path): First-shell Cd-O.

**Model 1b** (1 path): First-shell Cd-S.

**Model 2a** (2 paths): First-shell Cd-O and Cd-S.

**Model 2b** (2 paths): First-shell Cd-S and second-shell Cd-Cd.

**Model 3** (3 paths): First-shell Cd-O and Cd-S and second-shell Cd-Cd.

The three different fitting models were compared by employing F-tests based on crystallographic R-factors (square root of normalized sum of squared residuals calculated in Artemis) (Ravel and Newville, 2005) to determine whether a particular additional scattering shell leads to a significantly improved fit. The confidence level $\alpha$, that model $n+1$ is significantly better than model $n$, was calculated according to Downward et al. (2007), with $\alpha > 67\%$ indicating a significant improvement.

Model 2b was compared to model 1b for samples where EXAFS LCF showed a dominance of S-coordinated Cd and additionally suggested the presence of CdS (and therefore the presence of a second shell Cd-Cd path). Otherwise, model 2a was compared to model 1a or 1b, depending on the contribution of S-coordinated Cd according to EXAFS LCF. The results from model comparison are listed in Table B.6. In all oxic samples (2d EQ and 28d REOX), the fit with first-shell O+S was not significantly better than the fit with an O shell alone. In all reduced samples, the fit with first-shell O+S was significantly better than the fit with an S shell alone. Furthermore, in all 40-days reduced samples, the inclusion of a contribution from second-shell Cd at a distance corresponding to CdS gave a significantly better fit. The best fits and the corresponding EXAFS parameters are presented in Table B.7 and Figure B.4.
Table B.6. Normalized sum of squared residuals for different fit models and calculated probabilities $\alpha$ for the presence of a further O, S or Cd shell based on F-test (Downward et al., 2007). n is the number of independent points, m is the number of fit parameters, SSR is the normalized sum of squared residuals (R-factor) calculated in Artemis (Ravel and Newville, 2005). Values in bold indicate significant better fits. The best fit model for each sample is marked with *, the respective fit parameters are given in Table B.7.

<table>
<thead>
<tr>
<th>Sample</th>
<th>----- Model 1a/1b -----</th>
<th>----- Model 2a/2b -----</th>
<th>----- Model 3 -----</th>
<th>Model 2 vs. 1</th>
<th>Model 3 vs. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 14.29 m = 2</td>
<td>n = 14.29 m = 4</td>
<td>n = 14.29 m = 6</td>
<td>$\alpha$ (%)</td>
<td>$\alpha$ (%)</td>
</tr>
<tr>
<td>LS-HCu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2d EQ</td>
<td>O*</td>
<td>0.019</td>
<td>O+S</td>
<td>0.016</td>
<td>--</td>
</tr>
<tr>
<td>20d RED</td>
<td>O</td>
<td>0.037</td>
<td>S+O*</td>
<td>0.017</td>
<td>S+O+Cd</td>
</tr>
<tr>
<td>40d RED</td>
<td>S</td>
<td>0.110</td>
<td>S+O</td>
<td>0.016</td>
<td>S+O+Cd*</td>
</tr>
<tr>
<td>14d REOX</td>
<td>O*</td>
<td>0.024</td>
<td>O+S</td>
<td>0.024</td>
<td>--</td>
</tr>
<tr>
<td>28d REOX</td>
<td>O*</td>
<td>0.021</td>
<td>O+S</td>
<td>0.021</td>
<td>--</td>
</tr>
<tr>
<td>MS-HCu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2d EQ</td>
<td>O*</td>
<td>0.016</td>
<td>O+S</td>
<td>0.014</td>
<td>--</td>
</tr>
<tr>
<td>20d RED</td>
<td>S</td>
<td>0.121</td>
<td>S+O*</td>
<td>0.011</td>
<td>S+O+Cd</td>
</tr>
<tr>
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<td>0.027</td>
<td>S+Cd</td>
<td>0.020</td>
<td>S+Cd+O*</td>
</tr>
<tr>
<td>14d REOX</td>
<td>O</td>
<td>0.027</td>
<td>O+S*</td>
<td>0.021</td>
<td>O+S+Cd</td>
</tr>
<tr>
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<td>0.025</td>
<td>O+S</td>
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</tr>
<tr>
<td>HS-HCu</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>20d RED</td>
<td>S</td>
<td>0.052</td>
<td>S+O</td>
<td>0.025</td>
<td>S+O+Cd*</td>
</tr>
<tr>
<td>40d RED</td>
<td>S</td>
<td>0.031</td>
<td>S+Cd</td>
<td>0.012</td>
<td>S+Cd+O*</td>
</tr>
<tr>
<td>MS-LCu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2d EQ</td>
<td>O*</td>
<td>0.022</td>
<td>O+S</td>
<td>0.020</td>
<td>--</td>
</tr>
<tr>
<td>5d RED</td>
<td>S</td>
<td>0.167</td>
<td>S+O</td>
<td>0.023</td>
<td>S+O+Cd*</td>
</tr>
<tr>
<td>40d RED</td>
<td>S</td>
<td>0.023</td>
<td>S+Cd*</td>
<td>0.015</td>
<td>S+Cd+O</td>
</tr>
<tr>
<td>14d REOX</td>
<td>O*</td>
<td>0.042</td>
<td>O+S</td>
<td>0.034</td>
<td>--</td>
</tr>
<tr>
<td>28d REOX</td>
<td>O*</td>
<td>0.034</td>
<td>O+S</td>
<td>0.031</td>
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</tr>
</tbody>
</table>
Figure B.4. (a) \( k^3 \)-weighted Cd K-edge EXAFS spectra, (b) magnitudes and (c) real parts of the Fourier transforms of soil samples. Gray lines represent experimental data and dotted lines represent the best shell-fit (parameters in Table B.7). The Fourier transforms were calculated over the \( k \)-range 2.5-9.2 Å\(^{-1}\) (LS-HCu 2d EQ \( k \)-range of 2.5-7.5 Å\(^{-1}\)) with a sill width of \( dk = 2.5 \). Shell fits were performed in \( R \)-space (\( k \)-weights = 1+2+3) over the \( R + \Delta R \)-range 1.1-4.5 Å.
### Table B.7. EXAFS parameters determined by shell-fitting of Cd K-edge EXAFS sample spectra. $S_0^2$, $\Delta E_0$ and $\sigma^2$ were fixed to the values determined from shell-fits of Cd reference compounds (Table B.1).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Path Cd-O $^a$</th>
<th>Path Cd-S $^b$</th>
<th>Path Cd-Cd $^c$</th>
<th>CN</th>
<th>R (Å)</th>
<th>$\sigma^2$ (Å$^2$)</th>
<th>CN</th>
<th>R (Å)</th>
<th>$\sigma^2$ (Å$^2$)</th>
<th>CN</th>
<th>R (Å)</th>
<th>$\sigma^2$ (Å$^2$)</th>
<th>NSSR $^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS-HCu</td>
<td>2d EQ</td>
<td>5.03 ± 0.26</td>
<td>2.27 ± 0.01</td>
<td>0.01</td>
<td>0.019</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>20d RED</td>
<td>3.74 ± 0.26</td>
<td>2.27 ± 0.01</td>
<td>0.01</td>
<td>0.78 ± 0.28</td>
<td>2.53 ± 0.04</td>
<td>0.01</td>
<td>0.017</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>40d RED</td>
<td>1.91 ± 0.19</td>
<td>2.26 ± 0.01</td>
<td>0.01</td>
<td>2.05 ± 0.13</td>
<td>2.52 ± 0.01</td>
<td>0.003</td>
<td>3.11 ± 1.29</td>
<td>4.16 ± 0.04</td>
<td>0.016</td>
<td>0.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14d REOX</td>
<td>4.62 ± 0.20</td>
<td>2.26 ± 0.00</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>28d REOX</td>
<td>4.88 ± 0.20</td>
<td>2.27 ± 0.00</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>MS-Cu</td>
<td>2d EQ</td>
<td>4.94 ± 0.18</td>
<td>2.27 ± 0.00</td>
<td>0.01</td>
<td>0.016</td>
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</tr>
<tr>
<td></td>
<td>20d RED</td>
<td>2.07 ± 0.20</td>
<td>2.27 ± 0.01</td>
<td>0.01</td>
<td>1.98 ± 0.13</td>
<td>2.51 ± 0.01</td>
<td>0.003</td>
<td>0.011</td>
<td></td>
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<tr>
<td></td>
<td>40d RED</td>
<td>0.59 ± 0.23</td>
<td>2.26 ± 0.04</td>
<td>0.01</td>
<td>3.12 ± 0.16</td>
<td>2.52 ± 0.00</td>
<td>0.003</td>
<td>3.55 ± 1.58</td>
<td>4.14 ± 0.04</td>
<td>0.016</td>
<td>0.011</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>14d REOX</td>
<td>4.52 ± 0.33</td>
<td>2.28 ± 0.01</td>
<td>0.01</td>
<td>0.50 ± 0.36</td>
<td>2.49 ± 0.08</td>
<td>0.01</td>
<td>0.021</td>
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<tr>
<td></td>
<td>28d REOX</td>
<td>4.44 ± 0.20</td>
<td>2.27 ± 0.00</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>HS-HCu</td>
<td>20d RED</td>
<td>1.02 ± 0.26</td>
<td>2.24 ± 0.03</td>
<td>0.01</td>
<td>2.89 ± 0.18</td>
<td>2.52 ± 0.00</td>
<td>0.003</td>
<td>4.67 ± 1.76</td>
<td>4.15 ± 0.03</td>
<td>0.016</td>
<td>0.013</td>
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<tr>
<td></td>
<td>40d RED</td>
<td>0.42 ± 0.20</td>
<td>2.24 ± 0.05</td>
<td>0.01</td>
<td>3.33 ± 0.14</td>
<td>2.51 ± 0.00</td>
<td>0.003</td>
<td>6.23 ± 1.42</td>
<td>4.16 ± 0.02</td>
<td>0.016</td>
<td>0.008</td>
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<tr>
<td>MS-LCu</td>
<td>2d EQ</td>
<td>4.78 ± 0.20</td>
<td>2.27 ± 0.00</td>
<td>0.01</td>
<td>0.022</td>
<td></td>
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<tr>
<td></td>
<td>5d RED</td>
<td>2.39 ± 0.26</td>
<td>2.28 ± 0.01</td>
<td>0.01</td>
<td>1.86 ± 0.17</td>
<td>2.50 ± 0.01</td>
<td>0.003</td>
<td>3.14 ± 1.65</td>
<td>4.15 ± 0.05</td>
<td>0.016</td>
<td>0.016</td>
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</tr>
<tr>
<td></td>
<td>40d RED</td>
<td>3.59 ± 0.14</td>
<td>2.52 ± 0.00</td>
<td>0.003</td>
<td>4.18 ± 1.74</td>
<td>4.14 ± 0.04</td>
<td>0.016</td>
<td>0.015</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>14d REOX</td>
<td>4.34 ± 0.24</td>
<td>2.26 ± 0.01</td>
<td>0.01</td>
<td>0.042</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>28d REOX</td>
<td>4.64 ± 0.22</td>
<td>2.27 ± 0.01</td>
<td>0.01</td>
<td>0.034</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

$^a$ $S_0^2$ fixed to value form CaCO$_3$, $\Delta E_0$ and $\sigma^2$ fixed to average value from Cd(NO$_3$)$_2$. Cd-clay, Cd-goethite and Cd-carboxyl. $^b$ $S_0^2$ and $\Delta E_0$ fixed to values from CdS, $\sigma^2$ fixed value from CdS if LCF analysis revealed the presence of CdS, otherwise to 0.01 as given by Karlsson et al. (2006). $^c$ $S_0^2$, $\Delta E_0$ and $\sigma^2$ fixed to the values from CdS. $^d$ Normalized sum of squared residuals = $\sum_i (\text{data}_i - \text{fit}_i)^2 / \sum_i (\text{data}_i)^2$.

In Figure B.5, the coordination numbers for first-shell O and S and second-shell Cd from the best shell-fits are compared to the respective coordination numbers calculated from the fractions of Cd-carboxyl, Cd-thiol (pH9) and CdS derived from the LCF analysis of the respective soil EXAFS spectra, using the coordination numbers derived from shell-fits of the reference compounds (Table B.1). The results from both methods correlate well, especially for Cd-O. For Cd-S and Cd-Cd, coordination numbers determined by shell-fitting were consistently lower than the calculated values derived from LCF results. For the shell-fits of soil EXAFS spectra, the Debye-Waller factors for Cd-S and Cd-Cd were fixed to the values derived from crystalline CdS. As a
result, CN numbers may have been underestimated by shell-fitting if Cd-sulfide in the samples exhibited a higher disorder than the crystalline CdS reference. Considering that the fractions of S- and O-coordinated Cd derived from EXAFS LCF analysis were in line with fractions derived from the complementary LCF analysis of the soil XANES spectra (Figure B.7), the lower Cd-S and Cd-Cd coordination numbers derived from shell-fitting most likely indicated that S-coordinated Cd in the soil samples was present in more disordered form, most likely as nanoparticulate amorphous to poorly crystalline CdS or - to a certain degree - Cd coordinated by organic thiol groups or adsorbed to the surface of another metal-sulfide.

![Figure B.5](image)

**Figure B.5.** Comparison of coordination numbers (CN) for Cd-O (a), Cd-S (b) and Cd-Cd (c) scattering paths derived from shell fitting with theoretically calculated coordination numbers from LCF analysis.

**LCF analysis of Cd K-edge XANES spectra and comparison with EXAFS LCF results**

In addition to the 17 sample spectra analyzed by EXAFS LCF, we collected spectra of 7 more soil samples (MS-HCu: 10d RED, 15d RED; HS-HCu: 2d EQ, 10 d RED, 15d RED, 14d REOX; MS-LCu: 10d RED) and analyzed the whole dataset by XANES LCF. The additional spectra were recorded at room temperature. Spectra of MS-HCu 15dRED, HS-HCu 15dRED and MS-LCu 10dRED were collected at the DUBBLE beamline at the ESRF (equipped with a Si(111) double crystal
monochromator calibrated using a Cd foil (K-edge energy: 26711 eV); spectra collected at room temperature in fluorescence mode using a 9-element Ge solid state detector).

For XANES data extraction, $E_0$ was fixed to 26711 eV. Normalization was performed by subtracting a first-order polynomial function fit to the pre-edge region (−120 to −50 eV) and subsequently dividing by a second-order polynomial fit to the post-edge region (50 to 220 eV). LCF analysis was performed over the energy-range 26691 to 27744 eV using the same 3 references as used for EXAFS LCF analysis (Cd-carboxyl, Cd-thiol (pH9), CdS). The sample XANES and LCF spectra are shown in Figure B.6, the LCF parameters are listed in Table B.8.

---

**Figure B.6.** Normalized Cd K-edge XANES sample spectra (black lines) and LCF spectra (colored lines) of the LS-HCu (a), MS-HCu (b), HS-HCu (c) and MS-LCu (d) series. LCF was performed over the energy range 26691 to 27744 eV. The LCF results are summarized in Table B.8.
The comparison of XANES and EXAFS LCF results revealed consistent fractions of O- and S-coordinated Cd (Figure B.7), but individual Cd-thiol and CdS fractions varied significantly between these two methods (Table B.5 and Table B.8). Cd-thiol and CdS are hardly distinguishable by XANES spectroscopy, but have different EXAFS oscillations due to the contribution of first-shell O to the Cd-thiol and second-shell Cd to the CdS spectrum (see section B.1). The changes in the fraction of S-coordinated Cd species during the soil reduction period.

---

**Table B.8.** LCF results for the normalized XANES spectra. Note that Cd-thiol and CdS are hardly distinguishable by XANES spectroscopy and therefore not directly comparable to the EXAFS LCF results, but rather as sum of S-coordinated Cd species as shown in Figure B.7. Statistical fit uncertainties are given in brackets.

<table>
<thead>
<tr>
<th></th>
<th>Cd-carboxyl (%)</th>
<th>Cd-thiol (pH9) (%)</th>
<th>CdS (%)</th>
<th>NSSR (10⁻²)⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LS-HCu</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2d EQ</td>
<td>71 (± 0.0)</td>
<td>0 (± 0.0)</td>
<td>28 (± 0.0)</td>
<td>45</td>
</tr>
<tr>
<td>20d RED</td>
<td>47 (± 0.9)</td>
<td>40 (± 4.5)</td>
<td>12 (± 4.0)</td>
<td>10</td>
</tr>
<tr>
<td>40d RED</td>
<td>18 (± 1.0)</td>
<td>67 (± 5.2)</td>
<td>14 (± 4.7)</td>
<td>14</td>
</tr>
<tr>
<td>14d REOX</td>
<td>61 (± 1.0)</td>
<td>38 (± 5.3)</td>
<td>0.7 (± 4.7)</td>
<td>14</td>
</tr>
<tr>
<td>28d REOX</td>
<td>69 (± 1.2)</td>
<td>24 (± 6.3)</td>
<td>6.9 (± 5.6)</td>
<td>19</td>
</tr>
<tr>
<td><strong>MS-HCu</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2d EQ</td>
<td>70 (± 1.2)</td>
<td>25 (± 6.2)</td>
<td>5.7 (± 5.5)</td>
<td>18</td>
</tr>
<tr>
<td>10d RED</td>
<td>66 (± 0.0)</td>
<td>0 (± 0.0)</td>
<td>34 (± 0.0)</td>
<td>25</td>
</tr>
<tr>
<td>15d RED</td>
<td>20 (± 0.0)</td>
<td>80 (± 0.0)</td>
<td>0 (± 0.0)</td>
<td>159</td>
</tr>
<tr>
<td>20d RED</td>
<td>19 (± 1.2)</td>
<td>66 (± 6.2)</td>
<td>15 (± 5.5)</td>
<td>19</td>
</tr>
<tr>
<td>40d RED</td>
<td>0 (± 0.0)</td>
<td>68 (± 0.0)</td>
<td>32 (± 0.0)</td>
<td>25</td>
</tr>
<tr>
<td>14d REOX</td>
<td>62 (± 1.2)</td>
<td>27 (± 6.2)</td>
<td>11 (± 5.5)</td>
<td>18</td>
</tr>
<tr>
<td>28d REOX</td>
<td>64 (± 1.3)</td>
<td>14 (± 6.9)</td>
<td>22 (± 6.2)</td>
<td>23</td>
</tr>
<tr>
<td><strong>HS-HCu</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2d EQ</td>
<td>70 (± 0.0)</td>
<td>0 (± 0.0)</td>
<td>31 (± 0.0)</td>
<td>17</td>
</tr>
<tr>
<td>10d RED</td>
<td>59 (± 1.5)</td>
<td>23 (± 7.8)</td>
<td>16 (± 7.0)</td>
<td>30</td>
</tr>
<tr>
<td>15d RED</td>
<td>31 (± 2.8)</td>
<td>30 (± 14.9)</td>
<td>39 (± 13.2)</td>
<td>108</td>
</tr>
<tr>
<td>20d RED</td>
<td>0 (± 0.0)</td>
<td>75 (± 0.0)</td>
<td>25 (± 0.0)</td>
<td>20</td>
</tr>
<tr>
<td>40d RED</td>
<td>0 (± 0.0)</td>
<td>50 (± 0.0)</td>
<td>50 (± 0.0)</td>
<td>36</td>
</tr>
<tr>
<td>14d REOX</td>
<td>64 (± 1.8)</td>
<td>1.0 (± 9.6)</td>
<td>34 (± 8.5)</td>
<td>45</td>
</tr>
<tr>
<td><strong>MS-LCu</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2d EQ</td>
<td>65 (± 0.0)</td>
<td>34 (± 0.0)</td>
<td>0 (± 0.0)</td>
<td>8.0</td>
</tr>
<tr>
<td>5d RED</td>
<td>19 (± 0.8)</td>
<td>81 (± 4.4)</td>
<td>0 (± 3.9)</td>
<td>9.3</td>
</tr>
<tr>
<td>10d RED</td>
<td>2.4 (± 2.1)</td>
<td>33 (± 10.9)</td>
<td>64 (± 9.7)</td>
<td>60</td>
</tr>
<tr>
<td>40d RED</td>
<td>0 (± 0.0)</td>
<td>64 (± 0.0)</td>
<td>36 (± 0.0)</td>
<td>32</td>
</tr>
<tr>
<td>14d REOX</td>
<td>56 (± 1.3)</td>
<td>32 (± 6.6)</td>
<td>11 (± 5.9)</td>
<td>21</td>
</tr>
<tr>
<td>28d REOX</td>
<td>63 (± 1.4)</td>
<td>19 (± 7.5)</td>
<td>18 (± 6.7)</td>
<td>28</td>
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</tbody>
</table>

⁺Normalized sum of squared residuals = Σᵢ (dataᵢ − fitᵢ)² / Σᵢ (dataᵢ)².
were perfectly correlated with the changes in dissolved Cd concentrations, and dissolved Cd exhibited a linear decrease with increasing S-coordinated solid-phase Cd (Figure B.8).

Figure B.7. Correlation between XANES and EXAFS LCF fractions reported in Table B.5 and Table B.8. (a) Cd-carboxyl and (b) S-coordinated Cd (Cd-thiol (pH9) + CdS).

Figure B.8. (a) Time dependent transformation in Cd speciation. (b) Correlation between dissolved Cd concentrations and Cd-S XANES fraction (Cd-thiol (pH9) + CdS) during the reduction period.
B.5. Comparison between Cu and Cd solution dynamics

Figure B.9. Dissolved Cu and Cd concentrations during soil reduction and subsequent reoxidation. The respective periods of major sulfate reduction are marked as gray shaded areas in the left panels. Error bars indicate the standard deviation of experimental triplicates. (a) low-sulfate series with Cd and Cu spike (LS-HCu); (b) medium-sulfate series with Cd and Cu spike (MS-HCu); (c) high-sulfate series with Cd and Cu spike (HS-HCu); (d) medium-sulfate series with Cd spike (MS-LCu). Cu data for low-, medium- and high-sulfate series with Cd and Cu spike are from Fulda et al. (2013).
B.6. References


C. Supporting Information to Chapter 4

This chapter has been submitted with minor modifications for publication in *Environmental Science and Technology* as Supporting Information to: Fulda, B., Voegelin A., Maurer F., Kretzschmar R. Copper redox transformation and complexation by reduced and oxidized soil humic acid: 1. X-ray absorption spectroscopy study.
C.1. Information on XAS measurements and copper reference compounds

Sample preparation
For XAS analysis 95 mg of the samples with low or 30 mg of the samples with high copper loading were homogeneously mixed either with 10 mg boron nitride (BN, Merck) or 30 mg BN and 20 mg Licowax® C (Lonay, Switzerland), respectively. The powders were pressed into 10-mm pellets that were placed between Kapton® tape and stored under anoxic conditions until analysis.

Synthesis of copper reference compounds
Following Cu(II) and Cu(I) reference compounds were synthesized for X-ray absorption spectroscopy in this study:

Cu(II)-malate: 44 mg of Cu(CO$_3$)Cu(OH)$_2$ (Fluka, p.a.) were dissolved in 40 mL of a 100 mM L-malic acid (Sigma-Aldrich) solution (molar malate to Cu(I) ration 10:1) and adjusted with NaOH solution to pH 7. The solution was shock-frozen in liquid N$_2$ and freeze-dried.

Cu(II)-carboxyl: BioRex® 70 carboxylic resin (Bio-Rad) was washed five times with Milli-Q® water. 24 ml of a 1 mM Cu(NO$_3$)$_2$ solution were equilibrated for 24 h with 0.5 g of the wet resin (0.153 g dry weight) leading to a loading of ~10000 ppm. The pH value was adjusted with HCl to pH 7.4. Solution was decanted and residuum washed once with 25 ml Milli-Q® water. Resin was then ground in a agate mortar and dried in the glovebox vacuum chamber.

Cu(I)-thiol: Ambersep GT74® thiol resin (Supelco) was washed five times with Milli-Q® water. 22.9 ml of a 1 mM CuCl solution (0.1 M NaCl, 0.05 M HCl) were equilibrated for 24 h with 0.3 g of the wet resin (0.146 g dry weight) leading to a loading of ~10000 ppm. pH was adjusted with NaOH to pH 5.6. Solution was decanted and residuum washed once with 25 ml Milli-Q® water. Resin was then ground in a agate mortar and dried in the glovebox vacuum chamber. All redox sensitive steps were performed in a glovebox (O$_2$ < 1 ppm).
**C.1 Information on XAS measurements and copper reference compounds**

**Cu(I)-carbamate:** 20 mL of a 20 mM CuCl solution (0.1 M NaCl, 1 M HCl) was added slowly to 20 mL of a 200 mM sodium diethyldithiocarbamate trihydrate (\((\text{C}_2\text{H}_5)_2\text{NCSSNa 3H}_2\text{O}\), Fluka, p.a.) solution and adjusted with NaOH solution to pH 7. The black precipitate was centrifuged (30 min at 3566 g) and the residuum was shock-frozen in liquid N\(_2\) and freeze-dried in the glovebox vacuum chamber. All redox sensitive steps were performed in a glovebox (O\(_2\) < 1 ppm).

**X-ray absorption spectroscopy**

Spectra of H\(_2\)-untr\(_{\text{ox}}\) and L\(_1\)/H\(_1\)-untr\(_{\text{anox}}\) samples as well as spectra of the reference compounds Cu(II)-carboxyl, Cu(I)-thiol and Cu(I)-carb were collected at the XAS beamline at the Angströmquelle Karlsruhe (ANKA, Karlsruhe, Germany). All other samples, Cu(II)-malate and metallic copper were measured at beamline 11-2 at the Stanford Synchrotron Radiation Lightsource (SSRL, Stanford, USA). The measurements were performed either at about 4 K (11-2) or 15 K (XAS) using a closed cycle He-cryostat. The beamlines were equipped with Si(220) (11-2) and Si(111) (XAS) double crystal monochromators which were calibrated by setting the first inflection of the K absorption edge of a metallic Cu foil to 8979 eV. The monochromators were detuned to 75% (11-2) and 65% (XAS) of the maximal intensity to reduce higher harmonics in the beam. All samples spectra as well as spectra of Cu(II)-carboxyl and Cu(I)-thiol were collected in fluorescence mode using solid-state Ge detectors. Cu(II)-malate and metallic copper were measured in transmission mode. In addition, published spectra were used as references for data analysis. Their source, measurement conditions and structural information are listed in Table C.1.
Table C.1. Structural information, source and measurement conditions of Cu reference compounds used in the Cu K-edge X-ray absorption spectroscopy study.

<table>
<thead>
<tr>
<th>Reference name</th>
<th>Source (spectra/materials)</th>
<th>Structural information</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cu(II)-O/N</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu(II)-malate</td>
<td>synthesized (this study)</td>
<td>Coordination: 4 O, Distance (Å): 1.94</td>
<td>shell fit this study (Table C.5)</td>
</tr>
<tr>
<td></td>
<td>SSRL; 4 K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu(II)-BioReX®</td>
<td>synthesized (this study)</td>
<td>Coordination: 4 O, Distance (Å): 1.94</td>
<td>shell fit this study (Table C.5)</td>
</tr>
<tr>
<td></td>
<td>ANKA; 15K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu(II)-CopC</td>
<td>spectrum from Arnesano et al. (2003); 20K</td>
<td>Coordination: 2 N/O, Distance (Å): 1.97</td>
<td>Arnesano et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>2 His</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu(I)-S/Cl (2-coordinated)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu(I)-2S</td>
<td>spectrum from Pufahl et al. (1997); 10K</td>
<td>Coordination: 2 S, Distance (Å): 2.15</td>
<td>shell fit this study (Table C.5)</td>
</tr>
<tr>
<td>Cu(I)-OCl</td>
<td>spectrum from Fulton et al. (2000); 325°C</td>
<td>Coordination: 1 O, Distance (Å): 1.88</td>
<td>Fulton et al. (2000)</td>
</tr>
<tr>
<td>Cu(I)-2Cl</td>
<td>spectrum from Fulton et al. (2000); 325°C</td>
<td>Coordination: 2 Cl, Distance (Å): 2.13</td>
<td>Fulton et al. (2000)</td>
</tr>
<tr>
<td>Cu(I)-S/Cl (3/4-coordinated)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu(I)-3S</td>
<td>spectrum from Pufahl et al. (1997); 10K</td>
<td>Coordination: 3 S, Distance (Å): 2.26</td>
<td>shell fit this study (Table C.5)</td>
</tr>
<tr>
<td>Cu(I)-3Cl</td>
<td>spectrum from Brugger et al. (2007); RT</td>
<td>Coordination: 3 Cl, Distance (Å): 2.27</td>
<td>Brugger et al. (2007)</td>
</tr>
<tr>
<td>Cu(I)-GT74®</td>
<td>synthesized (this study)</td>
<td>Coordination: 2.9 S, Distance (Å): 2.24</td>
<td>shell fit this study (Table C.5)</td>
</tr>
<tr>
<td></td>
<td>ANKA; 15K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu(I)-carb</td>
<td>synthesized (this study)</td>
<td>Coordination: 2.8 S, Distance (Å): 2.27</td>
<td>shell fit this study (Table C.5)</td>
</tr>
<tr>
<td></td>
<td>ANKA; 15K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu(I)-O/N+S (3/4-coordinated)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu(I)-CopC</td>
<td>spectrum from Arnesano et al. (2003); 20K</td>
<td>Coordination: 1 His, Distance (Å): 1.95</td>
<td>Arnesano et al. (2003)</td>
</tr>
<tr>
<td>Cu(I)-mb</td>
<td>spectrum from Hakemian et al. (2005), 10K</td>
<td>Coordination: 2 N, Distance (Å): 2.03–2.05</td>
<td>Hakemian et al. (2005)</td>
</tr>
<tr>
<td>Metallic Cu</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu metal</td>
<td>Goodfellow 106-905-90</td>
<td>Coordination: 12 Cu, Distance (Å): 2.54</td>
<td>Wyckoff (1963)</td>
</tr>
<tr>
<td></td>
<td>SSRL; 4 K</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
XAS data processing

The spectra were processed using the analysis program Athena (Ravel and Newville, 2005). For data extraction, $E_0$ was fixed to 8988 eV. Background correction was performed by subtracting a first-order polynomial function fit to the pre-edge region (-150 to -40 eV) and subsequently dividing by a second-order polynomial fit to the post-edge region (60 to 490 eV). The background spline was adjusted using the Autobk algorithm ($R_{\text{bkg}} = 0.9; k$-weight = 3; $k$-range 0.5–11.3 Å$^{-1}$). EXAFS data over the $k$-range from 2.5 to 10.3 Å$^{-1}$ were Fourier-transformed using a Kaiser-Bessel apodization window ($dk = 2.5$ Å$^{-1}$).

Copper redox state and coordination environments of copper spiked HA samples were analyzed by linear combination fitting (LCF) of the normalized XANES spectra (energy range 8968–9018 eV) and the $k^3$-weighted EXAFS spectra ($k$-range 2.5–10.3 Å$^{-1}$) as described in the main part. Preliminary EXAFS LCF analysis showed that the sum of fitted fractions was often significantly less than 100%, and comparison with the results from XANES LCF analysis showed that the discrepancy could nearly exclusively be attributed to the Cu(I) fraction (data not shown). The reason may be destructive interferences between first-shell O/N and S/Cl signals (Figure 4.2a) which leads to substantial dampening or even extinction of the first-shell EXAFS oscillations in the higher $k$-range. The sum of the fitted fractions was therefore constrained to unity for EXAFS LCF analysis, which resulted in a very good agreement of the copper oxidation state derived from LCF analyses of EXAFS and XANES spectra (see next section, Figure C.1).
C.2. XANES and EXAFS LCF results

Figure C.1. Comparison of copper redox speciation derived from LCF analysis of XANES (Table C.2) and EXAFS (Table 4.2) spectra. The dashed grey lines indicate the ±10% interval around the 1:1 line.

Table C.2. Copper speciation in the samples based on LCF of Cu K-edge XANES spectra. Individual fitted fractions were constrained to the range 0 to 1, the sum of all fractions was not constrained.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cu(II) (%)</th>
<th>Cu(I) CN = 2 (%)</th>
<th>Cu(I) CN = 3-4 (%)</th>
<th>Cu(0) (%)</th>
<th>NSSR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2-untr</td>
<td>99 (carbox, copII)</td>
<td>-</td>
<td>1.9</td>
<td>-</td>
<td>6.1</td>
</tr>
<tr>
<td>H1-untr</td>
<td>38 (carbox, copII)</td>
<td>33 (2S, 2Cl)</td>
<td>29 (3Cl)</td>
<td>-</td>
<td>7.0</td>
</tr>
<tr>
<td>H2-red</td>
<td>18 (copII)</td>
<td>46 (OCl)</td>
<td>21 (thiol)</td>
<td>16</td>
<td>6.7</td>
</tr>
<tr>
<td>H1-red</td>
<td>4.8 (copII)</td>
<td>20 (OCl)</td>
<td>13 (thiol)</td>
<td>64</td>
<td>1.7</td>
</tr>
<tr>
<td>H2-red</td>
<td>95 (mal, carbox, copII)</td>
<td>-</td>
<td>5.2 (carb)</td>
<td>-</td>
<td>5.4</td>
</tr>
<tr>
<td>H1-red</td>
<td>95 (mal, carbox, copII)</td>
<td>-</td>
<td>5.8 (carb)</td>
<td>-</td>
<td>4.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cu(II) (%)</th>
<th>Cu(I) CN = 2 (%)</th>
<th>Cu(I) CN = 3-4 (%)</th>
<th>Cu(0) (%)</th>
<th>NSSR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2-untr</td>
<td>89 (mal, carbox, copII)</td>
<td>-</td>
<td>12 (carb, 3Cl)</td>
<td>-</td>
<td>3.3</td>
</tr>
<tr>
<td>L1-untr</td>
<td>72 (carbox, copII)</td>
<td>10 (2S)</td>
<td>18 (3Cl)</td>
<td>-</td>
<td>7.5</td>
</tr>
<tr>
<td>L2-red</td>
<td>17 (copII)</td>
<td>58 (2S, OCl)</td>
<td>21 (3Cl)</td>
<td>4.1</td>
<td>6.8</td>
</tr>
<tr>
<td>L1-red</td>
<td>16 (copII)</td>
<td>59 (2S, OCl)</td>
<td>22 (3Cl)</td>
<td>4.1</td>
<td>7.6</td>
</tr>
<tr>
<td>L2-red</td>
<td>92 (mal, carbox, copII)</td>
<td>-</td>
<td>8.8 (carb)</td>
<td>-</td>
<td>4.6</td>
</tr>
<tr>
<td>L1-red</td>
<td>89 (mal, carbox, copII)</td>
<td>-</td>
<td>12 (copl, carb)</td>
<td>-</td>
<td>3.8</td>
</tr>
</tbody>
</table>

\( ^a \) (mal)=Cu(II)-malate, (carbox)=Cu(II)-carboxyl, (copII)=Cu(II)-CopC protein; \(^b\) (2S)=Cu(I)-2S, (OCl)=Cu(I)-OCl, (2Cl)=Cu(I)-2Cl; \(^c\) (3Cl)=Cu(I)-3Cl, (thiol)=Cu(I)-thiol, (carb)=Cu(I)-carbamate, (copl)=Cu(I)-CopC protein; \(^d\) normalized sum of squared residuals = \( N \sum (data_{exp} - data_{calc})^2 / \sum (data_{exp})^2 \)
C.2 XANES and EXAFS LCF results

**Figure C.2.** Best XANES LCF for (a) H1-untr$_{anox}$ and (b) L1-red$_{anox}$ sample spectra, using only spectra of 2-fold (red line) or 3/4-fold (blue line) coordinated Cu(I) compounds in comparison to the best fit, using spectra of 2- and 3/4-fold coordinated Cu(I) reference compounds (black line, fit parameters in Table C.2). NSSR is the normalized sum of squared residuals $= \sum_i (\text{data}_{\text{exp}} - \text{data}_{i})^2 / \sum_i (\text{data}_{\text{exp}})^2$.

**Table C.3.** Comparison of 2- and 3/4-coordinated Cu(I) fractions (based on total Cu(I) amount) derived from XANES and EXAFS LCF.

<table>
<thead>
<tr>
<th></th>
<th>Cu(I) XANES</th>
<th></th>
<th>Cu(I) EXAFS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CN = 2 (%)</td>
<td>CN = 3-4 (%)</td>
<td>CN = 2 (%)</td>
</tr>
<tr>
<td>H2-untr$_{ox}$</td>
<td>-</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>H1-untr$_{anox}$</td>
<td>53</td>
<td>47</td>
<td>70</td>
</tr>
<tr>
<td>H2-red$_{anox}$</td>
<td>69</td>
<td>31</td>
<td>65</td>
</tr>
<tr>
<td>H1-red$_{anox}$</td>
<td>61</td>
<td>39</td>
<td>-</td>
</tr>
<tr>
<td>H2-reox</td>
<td>-</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>H1-reox</td>
<td>-</td>
<td>100</td>
<td>-</td>
</tr>
</tbody>
</table>

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>L2-untr$_{ox}$</td>
<td>-</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>L1-untr$_{anox}$</td>
<td>37</td>
<td>63</td>
<td>100</td>
</tr>
<tr>
<td>L2-red$_{anox}$</td>
<td>73</td>
<td>27</td>
<td>59</td>
</tr>
<tr>
<td>L1-red$_{anox}$</td>
<td>73</td>
<td>27</td>
<td>62</td>
</tr>
<tr>
<td>L2-red$_{ox}$</td>
<td>-</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>L1-red$_{ox}$</td>
<td>-</td>
<td>100</td>
<td>-</td>
</tr>
</tbody>
</table>

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Table C.4. Comparison of 2- and 3/4-coordinated Cu(I) fractions (based on total Cu(I) amount) derived from XANES and EXAFS LCF.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cu(II) (mmol kg(^{-1}))</th>
<th>Cu(I) (mmol kg(^{-1}))</th>
<th>Cu(0) (mmol kg(^{-1}))</th>
<th>Net electron transfer to Cu (mmol e(^{-}) kg(^{-1}))</th>
<th>e(\rightarrow)Cu / e(\rightarrow)HA(^{+}) ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2-red(_{anox})</td>
<td>1.3</td>
<td>5.9</td>
<td>0.3</td>
<td>6.5</td>
<td>1.2</td>
</tr>
<tr>
<td>H2-red(_{anox})</td>
<td>26</td>
<td>101</td>
<td>24</td>
<td>148</td>
<td>27</td>
</tr>
<tr>
<td>L1-red(_{anox})</td>
<td>1.2</td>
<td>6.0</td>
<td>0.3</td>
<td>-0.9</td>
<td></td>
</tr>
<tr>
<td>H1-red(_{anox})</td>
<td>7.1</td>
<td>48</td>
<td>95</td>
<td>88</td>
<td>16</td>
</tr>
</tbody>
</table>

* The amount of electrons transferred to HA during electrochemical reduction (0.55 mol kg\(^{-1}\)) was taken from Maurer et al. (2010).

C.3. TEM images of copper nanoparticle cluster

Sample preparation for TEM analysis

Reduced HA was reacted with Cu(I)-stock solution (5, 50 and 500 mmol kg\(^{-1}\)) as described for XAS sample preparation, equilibrated at pH 7.0 under anoxic conditions for one week and subsequently readjusted from pH 6.7 to pH 7.0 using 0.1 M NaOH. For transmission electron microscopy (TEM) analysis, sample materials were deposited onto carbon-coated nickel grids by ultracentrifugation (Kontron swing out rotor (TST 28.38), 2 h at 10600 g). Images were recorded in scanning mode using a high-angle annular dark-field detector (HAADF).

Cu(0) nanoparticles

TEM analysis of reduced HA reacted for 1 week with Cu(I) under anoxic condition, indicating the formation of metallic copper nanoparticles at copper loadings of 50 and 500 mmol kg\(^{-1}\) (Figure C.3) but not at a copper loading of only 5 mmol kg\(^{-1}\). The diameters of the spherical to oval particles were about 30–120 nm (on average larger at the higher copper loading). Particle cluster appeared to be more abundant at the higher copper loading.
Figure C.3. TEM images of metallic copper clusters formed after reacting Cu(I) with reduced HA for one week under anoxic conditions at copper loadings of 500 mmol kg$^{-1}$ (a, b) and 50 mmol kg$^{-1}$ (c-g). (h) Corresponding EDX spectra of background (EDX1) and Cu particles (EDX2 and EDX3).

C.4. Calculation of metallic copper saturation

Disproportionation equations

(1) \( \text{Cu}^{2+} + e^- \leftrightarrow \text{Cu}^+ \quad Eh = 0.153; \log K_1 = 2.59 \)

(II) \( \text{Cu}^+ + e^- \leftrightarrow \text{Cu}^0(s) \quad Eh = 0.521; \log K_2 = 8.83 \)

(II-I) \( 2 \text{Cu}^+ \leftrightarrow \text{Cu}^{2+} + \text{Cu}^0(s) \quad \log K_{\text{disp}} = \log K_2 - \log K_1 = 6.24 \)

\( \text{Cu}^0(s) \leftrightarrow 2\text{Cu}^+ - \text{Cu}^{2+} \quad \log K_{\text{sp}} = -6.24 \)

(a) \( 2 \log \{\text{Cu}^+\} - \log \{\text{Cu}^{2+}\} - \log K_{\text{sp}} < 0 \quad \) no disproportionation

(b) \( 2 \log \{\text{Cu}^+\} - \log \{\text{Cu}^{2+}\} - \log K_{\text{sp}} = 0 \quad \) equilibrium

(c) \( 2 \log \{\text{Cu}^+\} - \log \{\text{Cu}^{2+}\} - \log K_{\text{sp}} > 0 \quad \) disproportionation
Isotherm equations (Maurer et al., 2013)

\[ \log(\text{Cu}^{+}_{\text{ads}}) = a_1 + b_1 \cdot \log(\text{Cu}^+) \]
\[ \log(\text{Cu}^{2+}_{\text{ads}}) = a_2 + b_2 \cdot \log(\text{Cu}^{2+}) \]

with: \( a_1 = 4.64; b_1 = 0.64 \)
\( a_2 = 2.36; b_2 = 0.36 \)

approximation: \( \{\text{Cu}^{+}_{\text{ads}}\} \approx \text{Cu(I) XANES LCF}; \{\text{Cu}^{2+}_{\text{ads}}\} \approx \text{Cu(II) XANES LCF} \)

Gaussian error propagation

\[ SI = 2 \log(\text{Cu}^+) - \log(\text{Cu}^{2+}) - \log K_{sp} \]
\[ SI = 2 \cdot [(\log(\text{Cu}^{+}_{\text{ads}}) - a_1)/b_1] - [(\log(\text{Cu}^{2+}_{\text{ads}}) - a_2)/b_2] - \log K_{sp} \]

\[ \sigma_{SI}^2 = \left[2 \cdot 1/b_1 \cdot 1/((\text{Cu}^{+}_{\text{ads}}) \cdot \ln(10)) \cdot \Delta \text{Cu}^{+}_{\text{ads}}\right]^2 + \left[-1/b_2 \cdot 1/((\text{Cu}^{2+}_{\text{ads}}) \cdot \ln(10)) \cdot \Delta \text{Cu}^{2+}_{\text{ads}}\right]^2 \]

with: \( \Delta \text{Cu}^{+}_{\text{ads}} \) and \( \Delta \text{Cu}^{2+}_{\text{ads}} \) assumed as \( \pm 5\% \) of XANES LCF results

errors in \( a_1, a_2, b_1 \) and \( b_2 \) were not considered

Figure C.4. Calculation of Cu(0) saturation with respect to copper disproportionation. Activity of the free Cu\(^+\) and Cu\(^{2+}\) species was calculated using the adsorption isotherm reported in Part 2 of this study (Maurer et al., 2013). Error bars calculated with Gaussian error propagation. Open symbols indicate samples in which significant Cu(0) formation was observed (number in brackets give Cu(0) fraction determined by XANES LCF).
C.5. Results shell-fitting

Figure C.5. Shell-fits of $k^3$-weighted EXAFS spectra of copper model compounds (Cu(II)-carboxyl, Cd(I)-thiol and Cu(I)-carbamate) performed in $R$-space (results in Table C.5).

Table C.5. Shell-fitting results for $k^3$-weighted EXAFS spectra of copper model compounds (Cu(II)-carboxyl, Cd(I)-thiol and Cu(I)-carbamate) performed in $R$-space. Parameters in bold were fixed.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$S_0^2$</th>
<th>$\Delta E_0$ (eV)</th>
<th>path</th>
<th>CN</th>
<th>R (Å)</th>
<th>$\sigma^2$ (10$^{-3}$ Å$^2$)</th>
<th>NSSR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)-carboxyl</td>
<td>0.9</td>
<td>3.47 ±1.64</td>
<td>Cu-O</td>
<td>4</td>
<td>1.94 ±0.01</td>
<td>5.3 ±0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Cu(I)-thiol</td>
<td>1.0</td>
<td>7.69 ±2.48</td>
<td>Cu-S</td>
<td>2.9</td>
<td>2.24 ±0.02</td>
<td>8.4 ±2.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Cu(I)-carbamate</td>
<td>1.0</td>
<td>1.72 ±2.10</td>
<td>Cu-S</td>
<td>2.8</td>
<td>2.27 ±0.01</td>
<td>5.0 ±1.1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

$^a$ fixed to $S_0^2$ derived from first shell-fits of Cu(II)-malate, Cu(I)-2S/Cu(I)-3S (Table 4.3); $^b$ $k$-range = 2.5-9.5 ($dk = 2.5$), $R+\Delta R = 0.9-2$, Cu(II)-acetate (Wang et al., 2006) was used as structural model; $^c$ $k$-range = 2.5-9.5 ($dk = 2.5$), $R+\Delta R = 0.9-2.3$, Cu(II)-ethylenthioacetate (Ogawa et al., 1982) was used as structural model; $^d$ $k$-range = 2.5-11.7 ($dk = 2.5$), $R+\Delta R = 1.2-2.8$, Cu(II)-carbamate (Bonamico et al., 1965) was used as structural model; $^e$ normalized sum of squared residuals = $\sum (data_{exp} - data_{fit})^2 / \sum (data_{exp})^2$. 
Figure C.6. Shell-fits of $k^3$-weighted spectra of copper reference compounds (Cu(II)-malate, Cu(I)-2S and Cu(I)-3S) and the L1-red$_{anox}$ and H1-untr$_{anox}$ samples performed in $R$-space ($k$-range 2.5–9.5, $d k = 2.5$; $R + \Delta R$-range 0.9–2.3) using one Cu-O and Cu-S paths based on the structure of Cu(II)-ethylenethioacetate (Ogawa et al., 1982) (results in Table 4.3).
Table C.6. Comparison between the calculated O/N and S/Cl coordination numbers and distances in L1-red\textsuperscript{anox} and H1-untr\textsuperscript{anox} according to the EXAFS LCF fractions (Table 4.2) and results of the shell fit (Table 4.3).

<table>
<thead>
<tr>
<th>LCF fraction</th>
<th>CN (O/N)</th>
<th>R (O/N) (Å)</th>
<th>CN (S/Cl)</th>
<th>R (S/Cl) (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>L1-red\textsuperscript{anox}</strong></td>
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C.6. References


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