

# 1 Towards an Understanding of the Cd Isotope Fractionation during 2 Transfer from the Soil to the Cereal Grain

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13

## 14 Abstract

15 Cd in soils might be taken up by plants, enter the food chain and endanger human health. This  
16 study investigates the isotopic fractionation of major processes during the Cd transfer from  
17 soils to cereal grains. Thereto, soil, soil solution, wheat and barley plants (roots, straw and  
18 grains) were sampled in the field at three study sites during two vegetation periods. Cd  
19 concentrations and  $\delta^{114/110}\text{Cd}$  values were determined in all samples, soil solution  
20 compositions measured and dissolved Cd speciation modelled. Isotopic fractionation between  
21 soils and soil solutions ( $\Delta^{114/110}\text{Cd}_{20-50\text{cm-soil solution}} = -0.61$  to  $-0.68\text{‰}$ ) was nearly constant  
22 among the three soils. Cd isotope compositions in plants were heavier than in soils  
23 ( $\Delta^{114/110}\text{Cd}_{0-20\text{cm-plants}} = -0.55$  to  $-0.31\text{‰}$ ) but lighter than in soil solutions ( $\Delta^{114/110}\text{Cd}_{\text{soil solution-}$   
24  $\text{plants}} = 0.06$  to  $0.36\text{‰}$ ) and these differences correlated with Cd plant-uptake rates. In a  
25 preliminary model, desorption from soil, soil solution speciation, adsorption on root surfaces,  
26 diffusion, and plant uptake were identified as the responsible processes for the Cd isotope  
27 fractionation between soil, soil solution and plants whereat the first two processes dominated  
28 over the last three processes. Within plants, compartments with lower Cd concentrations

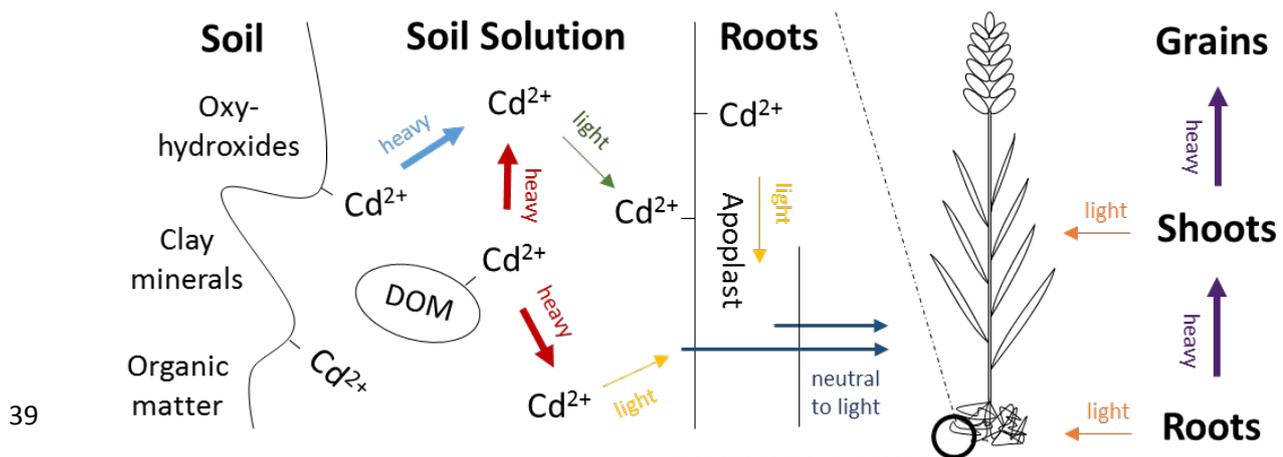
29 were enriched in light isotopes what might be a consequence of Cd retention mechanisms,  
30 following a Rayleigh fractionation, in which barley cultivars were more efficient than wheat  
31 cultivars.

32

33 **Capsule:** The isotopic fractionation between soil and soil solution is mainly driven by pool-size  
34 effects during desorption from the soil solid phase, which also controls Cd isotopes in plants,  
35 while the plant internal fractionation is controlled by Cd retention mechanisms which are  
36 more efficient in barley compared to wheat plants.

37

### 38 Graphical Abstract



### 40 Highlights

- 41 • The chemical characteristics of soil and solution does not affect isotope fractionation.
- 42 • Desorption and solution speciation control Cd isotopic composition of the plants.
- 43 • Cd retention controls the plant internal Cd isotope distribution in the plant.
- 44 • Barley plants are more effective in preventing Cd to be transferred to the grain.

45 **Keywords:** Cadmium, cereal, plant metal uptake, soil, soil solution

46

## 47 Introduction

48 Cadmium is a toxic element of major concern, which accumulates in many agricultural soils  
49 because of deposition from the atmosphere, mineral phosphate (P) fertilization, and the  
50 amendment of various materials, including compost, sewage sludge, waste water, and  
51 industrial by-products (Alloway and Steinnes, 1999). Within soils, Cd can desorb from solids  
52 into dissolved form, be taken up by crops, and thus enter the human food chain (Nagajyoti et  
53 al., 2010). Such uptake is undesired, as Cd is toxic for plants and humans and accumulates in  
54 human bodies (Godt et al., 2006). Even low Cd concentrations in edible plant parts can pose a  
55 risk for human health because the biological half-life of Cd is 10-30 years (Nordberg, 2009). To  
56 assure low Cd concentrations in crops, it is therefore important to better understand plant-  
57 uptake and plant-internal redistribution processes.

58 The path of Cd from soils to plant tissues involves the three steps of desorption, plant-uptake,  
59 and plant-internal redistribution. Each of these steps might involve isotopic fractionation.  
60 First, most Cd in soils is stored in the solid phase in various chemical forms (Wilcke et al.,  
61 2005) and only a minor part dissolves in the soil solution (Bruemmer et al., 1986) mainly  
62 determined by soil pH (Bruemmer et al., 1986; Christensen, 1984; Herms and Brümmer,  
63 1984; Sauvé et al., 2000). In soil solutions, Cd is either present as inorganic free Cd<sup>2+</sup> or forms  
64 a number of complexes (Nolan et al., 2003). Plants can alter their rhizosphere soils with the  
65 excretion of a wide range of organic (e.g., acids) and inorganic (e.g., H<sup>+</sup>) substances (Clemens  
66 et al., 2002; Welch, 1995). Consequently, the share of the plant available Cd might increase  
67 (Awad and Römheld, 2000; Grayston et al., 1997; Treeby et al., 1989; Dakora and Phillips,  
68 2002; Cancès et al., 2003). Second, non-specific uptake of Cd has been revealed for plants –  
69 with metal transporters that take up essential trace metals (Clemens et al., 2002; Perfus-  
70 Barbeoch et al., 2002; Clemens and Ma, 2016; Mendoza-Cózatl et al., 2011). Because  
71 rhizosphere pH can be altered by plants, soil Cd concentrations appear to mainly determine  
72 crop Cd concentrations (Clemens et al., 2002; Dakora and Phillips, 2002). Third, living cells  
73 developed strategies to avoid cytosolic Cd<sup>2+</sup> and prevent toxic effects; these strategies  
74 influence Cd concentrations in plant tissues and may be similar for bacteria and human cells  
75 (Prévéral et al., 2009). For Cd concentrations in grains of wheat and barley, xylem and phloem  
76 transport are important (Kubo et al., 2016; Chen et al., 2007).

77 To investigate Cd behavior in soils and its uptake into plants Cd stable isotopes might offer  
78 important information about biogeochemical processes. Cd in solution has been shown to be  
79 isotopically heavier than Cd in solid phases in leaching, sorption and coprecipitation processes  
80 ( $\Delta^{114/110}\text{Cd}_{\text{solid-fluid}} = -0.54$  to  $-0.24\%$ , Horner et al., 2011; Wasylenki et al., 2014; Zhang et al.,  
81 2016). Living cells were enriched in light isotopes probably through Cd sequestration into cell  
82 walls (Horner et al., 2013; McBride, 2002) and Cd-tolerant plants showed the same pattern  
83 ( $\Delta^{114/110}\text{Cd}_{\text{solution-plant}} = 0.30$  to  $0.46\%$ , Wei et al., 2016). In an earlier study of our group, pot  
84 experiments were done on two of the soils used here. Wheat plants were shown to be  
85 depleted in Cd and to get isotopically heavier from roots to straw and from straw to grains  
86 (Wiggenhauser et al., 2016). These fractionations were explained by Cd binding to S-ligands  
87 such as glutathione and phytochelatin; possibly with subsequent excretion or sequestration  
88 into vacuoles (Song et al. 2014; Dorcak and Krezel, 2003; Maret and Moulis, 2013; Mendoza-  
89 Cózatl et al., 2011; Prévéral et al., 2009). As a consequence, light isotopes were preferentially  
90 sequestered, which is also predicted by theoretical calculations (Wiggenhauser et al., 2016;  
91 Yang et al., 2015).

92 We formerly established Cd balances and calculated Cd stable isotopes fluxes at the three  
93 sites which are also presented in this paper (Imseng et al. 2018). Thereby, the anthropogenic  
94 impact on Cd concentrations in the soils could be assessed and important natural long-term  
95 processes were identified (Imseng et al. 2018). However, to understand Cd behavior and  
96 isotope fractionation in the soil-plant system, further research on Cd partitioning and Cd  
97 isotope fractionation between soil, soil solution and plants is necessary. Finally, by now, no  
98 field data about Cd isotope fractionation in wheat are available and data from other cereals  
99 are generally missing. To fill this research gap, Cd concentrations and Cd isotope compositions  
100 of soil, soil solutions and three plant compartments of wheat and barley were determined  
101 and Cd speciation in soil solution modelled. This knowledge will be important to further  
102 establish stable isotope tools for the investigation of Cd uptake into plants and thus to avoid  
103 Cd uptake into crops and the human food chain. Wheat was chosen because it is the most  
104 important food crop in Europe; barley as well is of economic importance and is often  
105 cultivated in crop rotation on the same soils as wheat (Leff et al. 2004). In this study, the  
106 following aims were aspired: (i) understand the influence of soil properties and Cd speciation  
107 in the soil solution on isotopic fractionation between soil and soil solution, (ii) assess the

108 relative importance of the five processes (#1) desorption from soil, (#2) soil solution  
109 speciation, (#3) adsorption on root surfaces, (#4) diffusion, and (#5) plant uptake for the  
110 overall isotope composition differences between soils and plants, and (iii) investigate the  
111 plant internal Cd redistribution in wheat and barley plants.

## 112 **Materials and Methods**

### 113 *Study Sites and Sampling*

114 The three study sites Oensingen (OE), Wiedlisbach (WI), and Nenzlingen (NE, Figure S1) as  
115 well as the sampling of soils and soil solutions were already described in a recent study  
116 (Imseng et al., 2018). Entire plants were harvested at full maturity, in summers 2014 and  
117 2015. The entire bulk root system was thereby sampled by removing a 20 cm-deep soil  
118 monolith with a surface area of 400 cm<sup>2</sup>. During the first season, winter wheat was grown at  
119 OE (*Triticum aestivum* L. cv. *Zinal*) and NE (*Triticum aestivum* L. cv. *Mulan*) and summer wheat  
120 at WI (*Triticum aestivum* L. cv. *Fiorina*). During the second season, winter barley was grown at  
121 OE (*Hordeum vulgare* L. cv. *Meridian*), WI (*Hordeum vulgare* L. cv. *Classic*) and NE (*Hordeum*  
122 *vulgare* L. cv. *Caravan*).

123

### 124 *Laboratory Analysis*

125 From all soil horizons, 0.05 M Ca(NO<sub>3</sub>)<sub>2</sub> extractable Cd was determined (Gray et al., 1999),  
126 followed by hot plate digestion with 40% HNO<sub>3</sub> to oxidize extracted organic complexes.  
127 Cadmium concentrations were measured with inductively-coupled plasma mass spectrometry  
128 (ICP-MS, 7700x, Agilent Technology, Waldbronn, Germany). Plants were separated into  
129 grains, straw (stem and leaves) and roots (Wiggenhauser et al., 2016). Grains and straw were  
130 dried at 60 °C for 48 h. Roots were rinsed with 6 mM NaNO<sub>3</sub> until no visible rhizosphere soil  
131 was left (Wiggenhauser et al., 2016) and dried at 60 °C for 48 h. The dried grains, straw and  
132 roots were weighed for the determination of the dry matter distribution, cut with a knife mill  
133 (GM 200, Retsch, Haan, Germany) and further ground with a planetary ball mill with agate  
134 beakers (PM 200, Retsch, Haan, Germany). Immediately after sampling of soil solutions, pH  
135 was determined in an aliquot that was afterwards discarded. For the soil solutions, the  
136 concentrations of major cations (Na<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup> and Ca<sup>2+</sup>) and anions (Cl<sup>-</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>,  
137 PO<sub>4</sub><sup>2-</sup> and SO<sub>4</sub><sup>2-</sup>) were measured by ion chromatography (IC, DX-120, ThermoFisher Scientific,  
138 Waltham, MA, USA) after filtration with 0.45 µm syringe filters (Whatman PVDF). The  
139 dissolved organic carbon (DOC) concentrations were analyzed with a vario TOC cube analyzer  
140 (Elementar Analysensysteme, Langenselbold, Germany) and total element concentrations (Al,

141 V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Cd, Ba, La, Ce, Pb, Th and U) were analyzed by ICP-  
142 MS.

143

#### 144 *Stable Isotope Measurements*

145 For the isotopic measurements, all samples were prepared in order to yield  $\geq 100$  ng of Cd for  
146 isotopic analysis (Wiggenhauser et al., 2016). As soil solution samples from OE and NE had  
147 very low Cd concentrations, between 1.1 and 5.3 L of soil solution were combined to acquire  
148 at least 10 ng Cd for isotopic analysis and evaporated prior to digestion. Because of the low  
149 plant Cd concentrations, wheat and barley samples were pre-digested in a microwave oven  
150 (ETHOS, MLS, Leutkirch, Germany). Depending on the Cd concentration, 0.5-3.0 g of sample  
151 were digested in 10 mL HNO<sub>3</sub> (69%) at 200°C for 0.5 h, transferred to Savillex® beakers  
152 (Savillex Corporation, Eden Prairie, MN, USA) and evaporated to dryness on a hot plate (120  
153 °C). Evaporated water samples, pre-digested plant samples and between 0.1 to 0.3 g of soil  
154 samples were digested on the hot plate (120 °C, 48 h) in 3 mL HNO<sub>3</sub> (69%), 1 mL H<sub>2</sub>O<sub>2</sub> (30%)  
155 and 2 mL HF (40%). The digests were evaporated to dryness, re-dissolved 4 mL aqua regia  
156 (120 °C, 48 h) and dried again. The samples were then dissolved in 5 M HCl and Cd  
157 concentrations were determined on small solution aliquots by ICP-MS. A <sup>111</sup>Cd/<sup>113</sup>Cd double  
158 spike solution was added to the samples to obtain a ratio of spike-to sample-derived Cd of  
159  $\sim 1.2$  and equilibrated for 24 h. Cadmium was then separated from the sample matrix using a  
160 three-stage column chemistry that employs both anion exchange and extraction  
161 chromatography (Murphy et al., 2016; Xue et al., 2012) This was followed by a liquid-liquid  
162 extraction step for further sample cleanup (Murphy et al., 2016).

163 The Cd isotope compositions were then determined on a Nu-Plasma HR multiple collector  
164 inductively-coupled plasma mass spectrometer (MC-ICP-MS, Nu Instruments Ltd, Wrexham,  
165 UK) at the Imperial College London MAGIC Laboratories. The isotope compositions of all  
166 samples were measured using standard-sample bracketing and using the added double-spike  
167 for correction of instrumental mass bias (Murphy et al., 2016; Xue et al., 2012). The total  
168 procedural Cd blank (n=11) for the isotopic measurements ranged from 110 to 1011 pg. This  
169 is equivalent to less than 2.5% of the smallest indigenous Cd mass among the samples, whilst  
170 the typical blank proportion was about 0.4%. Hence, no blank corrections were required for

171 the isotopic data. Several standard reference materials (SRMs) were analyzed together with  
172 the samples for quality control and the results show good agreement with previously  
173 published values (Table S3). The double-spike method also yields precise Cd concentrations  
174 (Ripperger and Rehkämper, 2007). For the SRMs, the measured Cd concentrations were  
175 slightly lower than the certified values but our data are in line with the results of other  
176 recent studies (Goix et al., 2011; Jochum et al., 2005; Wiseman et al., 2013).

177

### 178 *Modelling and Calculations*

179 Cd speciation in the soil solution was calculated with Visual MINTEQ (Table S1). The pH,  
180 cation, anion, total element and dissolved organic matter concentration from the analyzed  
181 soil solution samples were used as input data. Ionic strength was calculated based on the ion  
182 and element concentrations, by calculating the sum of all positive charges in solution (cation  
183 concentration x cation charge). Dissolved organic matter (DOM) concentrations were inferred  
184 from the measured dissolved organic carbon concentrations (DOC), using a converting factor  
185 DOM:DOC of 2:1 (Pribyl, 2010). Cadmium speciation was modelled with two scenarios,  
186 because it has been reported that the DOM composition is an important source of  
187 uncertainty (Groenenberg et al., 2010). In the min-HS and max-HS scenario, DOM consisted to  
188 20% and 60% of humic hydrophobic substances (HS), respectively, in line with reported values  
189 (Groenenberg et al., 2010; Ren et al., 2015). The remaining mass fractions were assumed to  
190 consist of hydrophilic organic substances. This fraction can be neglected in the models  
191 because it was shown that they do not influence Cd speciation (Groenenberg et al., 2010; Ren  
192 et al., 2015). In both scenarios, humic substances were subdivided into fulvic (FA, 90%) and  
193 humic (HA, 10%) acids (Groenenberg et al., 2010; Ren et al., 2015). DOM in the min-HS  
194 scenario therefore consists to 2% of humic acids, 18% of fulvic acids and 80% of hydrophilic  
195 organic substances whilst in the max-HS scenario it is comprised of 6% humic acids, 54% fulvic  
196 acids and 40% hydrophilic substances.

197 To calculate the expected Cd stock in plants, the crop transpiration ( $T_c$ ) was calculated by  
198 correcting the reference evapotranspiration  $ET_0$  with the basal crop coefficient ( $K_{cb}$ , Allen,  
199 1998). To this end,  $ET_0$  was calculated according the FAO Penman-Monteith method (Allen,  
200 1998) whilst  $K_{cb}$  was obtained from the meteorological (wind speed and relative humidity)

201 and agricultural (crop type, growth stage and crop heights) data provided by the Federal  
202 Office of Meteorology and Climatology (MeteoSwiss, 2015) and derived from the biweekly  
203 observations at the study sites, respectively. For the meteorological data, the stations in  
204 Wynau (for OE and WI) and Basel/Binningen (for NE) were chosen (Figure S1, MeteoSwiss,  
205 2015).

206 The expected Cd stock of plants was determined by multiplying the biweekly measured soil  
207 solution Cd concentration with the biweekly data for the calculated crop transpiration of  
208 wheat per area. The concept of the expected Cd stock in plants assumes that the plants do  
209 not alter their rhizosphere soil and take up the soil solution with its measured Cd  
210 concentration. The actual Cd stock in wheat and barley plants was calculated from the  
211 measured Cd concentration in plant parts (root, straw and grains) and the farmers' data on  
212 the dry matter of harvested straw and grains per area. The dry matter of roots per area was  
213 derived from the contribution of root-associated dry matter to that of the harvested plants.  
214 Because wheat and barley plants were only harvested once per site, standard deviations  
215 could not be calculated for the dry matter distribution. Hence, standard deviations for dry  
216 matter were assumed to be the same as in a previous pot study, partly conducted on the  
217 same soils (Wiggerhauser et al., 2016).

218 The Cd isotope compositions of the samples are reported relative to the NIST 3108 Cd isotope  
219 reference material using a  $\delta$  notation based on the  $^{114}\text{Cd}/^{110}\text{Cd}$  ratio (Equation 1).

220 **Equation 1:** 
$$\delta^{114/110}\text{Cd} = \left[ \frac{(^{114}\text{Cd}/^{110}\text{Cd})_{\text{sample}}}{(^{114}\text{Cd}/^{110}\text{Cd})_{\text{NIST3108}}} - 1 \right] \cdot 1000$$

221 The  $\Delta^{114/110}\text{Cd}$  values, which denote the apparent isotopic fractionation between two sample  
222 types (e.g., between soil and soil solution) were calculated according to Equation 2 with an  
223 error propagation according to Kusonwiriawong et al. (2017).

224 **Equation 2:** 
$$\Delta^{114/110}\text{Cd}_{\text{soil-soilsolution}} = \delta^{114/110}\text{Cd}_{\text{soil}} - \delta^{114/110}\text{Cd}_{\text{soilsolution}}$$

225

226 The isotopic compositions of whole plants or whole-year soil solutions are comprised of  
227 several fractions (e.g., wheat plant = roots + straw + grains). The average isotopic composition

228 was calculated according to Equation 3 using an error propagation according to  
229 Kusonwiriawong et al. (2017).

230 **Equation 3:** 
$$\delta^{114/110}Cd = \frac{\sum_{f=1}^n \delta^{114/110}Cd_f \cdot mCd_f}{\sum_{f=1}^n mCd_f}$$

231  $\delta^{114/110}Cd$ : isotopic ratio of the whole plant

232  $\delta^{114/110}Cd_f$ : isotopic ratio of a plant compartment

233  $mCd_f$ : Cadmium mass of a compartment

234

235

236 The variability of the isotope compositions between the different plants was calculated by  
237 comparing the  $\Delta^{114/110}Cd_{\text{grains-roots}}$  for each site and plant. The statistical significance was  
238 subsequently calculated using ANOVA with a level of significance of  $p < 0.05$ .

239

## 240 Results

241 At all three sites, the extractable soil Cd concentrations decreased with depth (Figure S2a). On  
242 average, they were highest at WI (18.1  $\mu\text{g kg}^{-1}$ ), followed by NE (5.2  $\mu\text{g kg}^{-1}$ ) and OE (4.3  $\mu\text{g kg}^{-1}$ ).  
243 All other considered soil properties including total soil Cd concentrations and pH were  
244 published in a recent study (Imseng et al., 2018) and are shown in Table S2. Also the bulk  
245 plant and soil solution Cd isotope analyses are already reported there. So in this study we  
246 focus on a higher temporal variation, different plant compartments and a more detailed  
247 chemical characterization of the soil solution. The averaged pH of the biweekly sampled soil  
248 solutions was lowest at WI (6.0) and higher at OE (7.8) and NE (7.6, Figure 1, Table S1). In line  
249 with soil pH, the average soil solution Cd concentration at WI (156  $\text{ng L}^{-1}$ ) exceeded the values  
250 at NE (11.3  $\text{ng L}^{-1}$ ) and OE (3.2  $\text{ng L}^{-1}$ ), by one or two orders of magnitude, respectively. In  
251 contrast to pH and dissolved Cd concentrations, the DOC concentrations (2.6 to 5.6  $\text{mg L}^{-1}$ )  
252 and ionic strengths (1.7 to 11.8  $\text{mmol L}^{-1}$ ) displayed only limited variability among the three  
253 sites. Modelling of Cd speciation revealed that dissolved Cd was either bound to DOM or  
254 present as free  $\text{Cd}^{2+}$  whilst other species contributed less than 5% to the dissolved Cd  
255 concentration (Table S1). On average, the share of free  $\text{Cd}^{2+}$  was higher in the min-HS than in  
256 the max-HS scenario at OE (38 and 13%), WI (70 and 41%) and NE (49 and 20%). Furthermore,  
257 the proportion of free  $\text{Cd}^{2+}$  to the total Cd concentrations typically increased with decreasing  
258 pH (Figure 1).

259 The Cd concentrations of wheat and barley plants at all sites decreased from roots (208-465  
260  $\text{ng g}^{-1}$ ) to straw (65-247  $\text{ng g}^{-1}$ ) to grains (17-98  $\text{ng g}^{-1}$ , Figures S3a & S3b). Among the sites,  
261 the Cd concentrations of the whole wheat and barley plants correlated with the Cd  
262 concentrations of the solid soils (Figure S4), but not of the soil solution. The Cd  
263 concentrations of individual wheat and barley plant parts correlated with the soil Cd  
264 concentrations (Figure S4), except for barley roots, where the concentrations at WI (221  $\text{ng g}^{-1}$ )  
265 were higher than at OE (431  $\text{ng g}^{-1}$ ). Straw contributed most to the dry matter (54 to 60%)  
266 of wheat plants at all three sites (Figures S3c and S3d) whilst grains provided most of the dry  
267 matter for barley plants (56 to 60%). For both plants and at all sites, the roots contributed the  
268 smallest proportion to dry matter. Moreover, the dry matter distribution among the plant  
269 parts of both crops was similar for the three sites, despite growing of different wheat and  
270 barley cultivars at the three sites, respectively. For both plants, most of the Cd was stored in

271 straw (54 to 80%, Figures S3e and S3f), followed by grains (15 to 28%) and roots (5 to 24%).  
272 An exception to this are the barley roots from WI as these contained more Cd (at 24%) than  
273 barley grains (21%). Overall, Cd was distributed more uniformly in barley than in wheat, as the  
274 latter displays larger differences in Cd content between roots, straw and grains. Crop  
275 transpiration ( $T_c$ ) was calculated for the complete wheat-cultivation period and similar values  
276 of 415, 530, and 507 L m<sup>-2</sup> were determined for OE, WI and NE, respectively. The expected Cd  
277 stock in wheat was 0.07x10<sup>4</sup>, 3.54x10<sup>4</sup>, and 0.24x10<sup>4</sup> ng m<sup>-2</sup> at OE, WI and NE, respectively. In  
278 contrast, the measured Cd stock was about two orders of magnitude higher both at OE  
279 (15.70x10<sup>4</sup> and 3.85x10<sup>4</sup> ng m<sup>-2</sup> for wheat and barley, respectively) and NE (18.95x10<sup>4</sup> and  
280 9.05x10<sup>4</sup> ng m<sup>-2</sup>) but close to the expected value at WI (5.94x10<sup>4</sup> and 4.85x10<sup>4</sup> ng m<sup>-2</sup>).

281 The isotopic compositions of bulk soils (Imseng et al., 2018), soil solutions (Imseng et al.,  
282 2018), and the Ca(NO<sub>3</sub>)<sub>2</sub> extracts of soils (Wiggenhauser et al., 2016) were published in recent  
283 articles. Compared to the soils, the whole wheat and barley plants were enriched in heavy  
284 isotopes and the isotopic fractionation between the soil and whole plants showed only  
285 limited variability between the sites for both wheat ( $\Delta^{114/110}\text{Cd}_{\text{soil}(0-20\text{cm})-\text{wheat}} = -0.31$  to -  
286 0.46‰) and barley ( $\Delta^{114/110}\text{Cd}_{\text{soil}(0-20\text{cm})-\text{barley}} = -0.51$  to -0.55‰, Figure 2a). A comparison of the  
287 isotopic data for soil solutions and whole plants thereby indicates that light Cd isotopes are  
288 more strongly enriched in wheat ( $\Delta^{114/110}\text{Cd}_{\text{soil solution-wheat}} = 0.20$  to 0.36‰) and barley  
289 ( $\Delta^{114/110}\text{Cd}_{\text{soil solution-barley}} = 0.06$  to 0.18‰) than in the soil solutions. Within wheat and barley  
290 plants of all three sites, the  $\delta^{114/110}\text{Cd}$  values increased systematically from roots (-0.05 to  
291 0.20‰, Figure 2b) to straw (0.09 to 0.51‰) and grains (0.47 to 1.32‰). The Cd isotope  
292 compositions of the plant parts were thereby significantly more variable in barley  
293 ( $\delta^{114/110}\text{Cd}_{\text{barley plant parts}} = 0.06$  to 1.32‰) than in wheat ( $\delta^{114/110}\text{Cd}_{\text{wheat plant parts}} = -0.05$  to  
294 0.77‰), whilst Cd was more uniformly distributed in the former.

295

## 296 Discussion

### 297 *Cd in Soil and Soil Solution*

298 The pH of soils and soil solutions mainly controlled the extractable Cd, dissolved Cd in soil  
299 solutions, and the share of free Cd<sup>2+</sup> in the same solutions and thus the plant available Cd.  
300 The DOM concentration and composition had a minor influence on the plant available Cd.  
301 First, a comparison of the pH as well as the total and extractable Cd concentration data for  
302 the three sites at each depth reveals that the extractable Cd, similar to the Cd concentrations  
303 in soil solutions, was mainly influenced by soil pH and not by total Cd concentrations (Figure  
304 S2). The extractable Cd concentrations decreased with depth at all three sites. In the upper  
305 soil horizons, Cd may originate from decomposed organic matter, mineral fertilizers and  
306 manure, while in deeper soil horizons, Cd may be increasingly derived from unweathered  
307 minerals (Schweizer et al. 2018; Loganathan and Hedley, 1996; Degryse et al., 2003; de Groot  
308 et al., 1998). Second, the soil solution pH not only correlated negatively with soil solution Cd  
309 concentrations (Figure 1a, Table S1), but also affected the Cd speciation in soil solutions with  
310 a trend to higher proportions of free (plant available) Cd<sup>2+</sup> at lower pH values (Figures 1b and  
311 1c, Table S1). WI was the site with highest fractions of free Cd<sup>2+</sup> probably because the soil has  
312 the lowest pH and the highest dissolved Cd concentrations. For OE and NE, additional  
313 calculations with Visual MINTEQ (data not shown) revealed that the share of free Cd<sup>2+</sup> should  
314 increase with both lower pH and higher dissolved Cd concentrations, when all other solution  
315 properties are kept constant. Finally, the DOM content and composition impacts the Cd  
316 speciation. In the min-HS scenario (20% of DOM are humic substances), more of the soil  
317 solution Cd is present as free Cd<sup>2+</sup> than in the max-HS scenario (60% humic substances). This  
318 is reasonable because humic substances, which consist mainly of fulvic acids, are the main  
319 cation sorbent in soil solutions (Groenenberg et al., 2010; Ren et al., 2015). Hence, increasing  
320 the DOM concentrations at a constant contribution of humic substances to DOM will  
321 decrease the share of free Cd<sup>2+</sup>.

322 The average isotopic fractionation between the soil solutions and the soils varied little among  
323 the sites with a consistent enrichment of the heavy isotopes in the former ( $\Delta^{114/110}\text{Cd}_{\text{soil}(20-}$   
324  $50\text{cm})\text{-soil solution} = -0.61$  to  $-0.68\text{‰}$ , Imseng et al., 2018). The fractionation was thereby not  
325 affected by any other property of the soil solid phase (Cd concentration, pH, cation exchange

326 capacity (CEC), organic matter concentration, texture, Imseng et al., 2018) or soil solution (Cd  
327 concentration, pH, Cd speciation, DOM concentration, Table S1). Our data are in line with the  
328 results of comparable studies, which showed an enrichment of heavy isotopes in the solution  
329 phase, after simulated and natural weathering (Zhang et al., 2016), adsorption to Mn  
330 oxyhydroxides (Wasylenki et al., 2014) and co-precipitation with calcite (Horner et al., 2011).

331 The isotope fractionation pattern of Cd between solid and solution phases can be explained  
332 by bond lengths between Cd and its surrounding atoms (Wasylenki et al. 2014). Equilibrium  
333 isotope fractionation is associated with an enrichment of the heavier isotopes in the phase  
334 with the shorter and stiffer bonds (Wiederhold, 2015). EXAFS studies have revealed that  
335 distances between Cd and the neighboring oxygen atoms are slightly shorter for aqueous Cd  
336 (2.27 to 2.28 Å) than for Cd carbonates (2.28 Å), Cd bound to goethite or humic acids (2.28 to  
337 2.30 Å), Cd(NO<sub>3</sub>)<sub>2</sub> (2.30 Å), Cd bound to  $\gamma$ -manganite or cellulose (2.31 Å) and Cd bound to  
338 pectin or malate (2.32 Å) (Ohtaki and Johansson, 1981; Vasconcelos et al., 2008; Fulda et al.,  
339 2013; Bochatay et al., 2000; Isaure et al., 2015; Karlsson et al., 2005). Even longer distances  
340 have been reported for bonds between Cd and sulfur atoms of organic matter (2.51 to 2.56 Å,  
341 Fulda et al., 2013; Isaure et al., 2015; Karlsson et al., 2005). These findings explain why several  
342 studies consistently reported enrichments of heavy isotopes in solutions relative to various  
343 solid phases (Horner et al., 2011; Imseng et al., 2018; Wasylenki et al., 2014; Zhang et al.,  
344 2016). Only a few EXAFS studies found Cd-O bonds of adsorbed Cd to be shorter than those of  
345 aqueous Cd, namely Cd bound to mineral interlayer or surface oxides (2.16 to 2.24 Å), Cd-  
346 glutathione complexes (2.26 Å), Cd bound to kaolinite (2.26 to 2.29 Å) and Cd bound to  
347 cysteine or clay minerals (2.27 Å). The enrichment of light isotopes indicates that these  
348 sorbents played a minor role for the Cd adsorption in our soils (Malferrari et al., 2007; Isaure  
349 et al., 2015; Vasconcelos et al., 2008; Sajidu et al. 2008; Fulda et al., 2013).

350 In contrast, bonds between Zn (2.06 to 2.11 Å) or Cu (four 1.97 Å and two 2.38 Å) and O for  
351 octahedral aqueous species are consistently longer than those for other  
352 octahedrally/tetrahedrally complexed Zn (1.94 to 2.06 Å) and Cu (1.85 to 2.12) species (Fulton  
353 et al., 2000; Kuzmin et al., 1997; Waychunas et al. 2002; Juillot et al., 2008; Nelson et al.,  
354 2017; Peacock and Sherman, 2004). Consequently, Zn and Cu adsorbed to humic substances,  
355 silicates, oxyhydroxides, and calcite have been reported as isotopically heavier than the  
356 aqueous Cu and Zn species (Bigalke et al., 2010; Jouvin et al., 2009; Ryan et al., 2014; Nelson

357 et al., 2017; Balistrieri et al., 2008; Bryan et al., 2015; Juillot et al., 2008; Pokrovsky et al.,  
358 2008; Dong and Wasylenki, 2016). The reverse bond length pattern between aqueous and  
359 adsorbed species for Zn and Cu in comparison to Cd are also reflected in the solution-solid  
360 phase partitioning coefficients ( $K_d$  values) for humic acids and Fe-oxides which indicate an  
361 increasing cation selectivity of the solid phases in the order  $\text{Ca} \ll \text{Cd} < \text{Zn} \ll \text{Cu}$  (Smolders  
362 and Mertens, 2013). Accordingly, we would expect that aqueous Ca, like Cd, is also  
363 isotopically heavier than the adsorbed Ca and this has been indeed shown in former studies  
364 (Hindshaw et al., 2013; Ockert et al., 2013).

365 The similar direction and extent of isotope fractionation between soil and soil solution for the  
366 three sites suggests that the Cd sorption mechanism was similar among the three soils. It is  
367 presumably dominated by inner-sphere adsorption to organic matter and hydroxides and not  
368 by outer-sphere adsorption to clay minerals. It was reported, that clay minerals are by a  
369 factor of 5 more selective for  $\text{Cd}^{2+}$  than for  $\text{Ca}^{2+}$  (Farrah et al. 1980). However, the soil  
370 solution Cd concentrations were 5 (WI), 7 (NE) and 8 (OE) orders of magnitude below the soil  
371 solution Ca concentrations. Therefore, outer-sphere adsorption on clay minerals presumably  
372 played an insignificant role for the Cd adsorption in our soils. This is further confirmed by the  
373 fact that isotopic fractionation did not differ between the three sites despite  $\text{CEC}_{\text{eff}}$  and pH  
374 were higher at OE and NE than at WI (Imseng et al., 2018). Hence, Cd was most probably  
375 bound to organic matter and hydroxides as inner-sphere complex (Smolders and Mertens,  
376 2013). Adsorption to organic matter, was reported to dominate in soils with  $\text{pH} < 6.5$  (i.e. WI)  
377 whereas adsorption to Fe-oxides becomes more important at  $\text{pH} > 6.5$  (i.e. OE and NE,  
378 Buekers et al., 2008). Hence, organic matter and Fe-oxides seem to be similar in their Cd  
379 isotope fractionation.

380 The isotope compositions of the  $\text{Ca}(\text{NO}_3)_2$  extractable Cd, determined in a pot study with the  
381 same soils of OE and WI (Wiggenhauser et al., 2016), pointed out that a higher ionic strength  
382 enhanced the Cd desorption and caused an isotopic pool-size effect by shifting the Cd isotope  
383 compositions in the dissolved fraction towards the Cd isotope compositions of the soils.  
384 Similar to the soil solution, the  $\text{Ca}(\text{NO}_3)_2$  extractable Cd was isotopically heavier than the Cd of  
385 the solid soils ( $\Delta^{114/110}\text{Cd}_{\text{soil}(0-20\text{cm})-\text{Ca}(\text{NO}_3)_2 \text{ extract}} = -0.44$  and  $-0.31\text{‰}$ ). However, the enrichment  
386 of heavy isotopes in the extracted Cd was less pronounced than in the Cd from soil solutions  
387 (Figure 2). It was shown for Zn that higher ionic strength is associated with a more

388 pronounced enrichment of heavy isotopes on surfaces, most probably because the competing  
389 ions occupied the outer-sphere adsorption sites such that Zn adsorbed more specifically as  
390 inner-sphere complex with shorter and stiffer bonds (Bryan et al., 2015; Nelson et al., 2017).  
391 The ionic strength of our extracts ( $\sim 0.1$  M) was about one order of magnitude higher  
392 compared to the soil solutions ( $\sim 0.01$  M). Hence, the  $\text{Ca}^{2+}$  ions of the 0.05 M  $\text{Ca}(\text{NO}_3)_2$   
393 extraction solutions most likely competed with Cd for the inner-sphere sorption sites of  
394 organic matter and hydroxides. This is reasonable, given that the  $K_d$  values for Ca are only 2 to  
395 4 orders of magnitude smaller than those of Cd but the Ca concentrations of the extraction  
396 solutions exceeded those of Cd in the soil solutions by 8 to 10 orders of magnitude. This  
397 suggests that the Ca ions of the extraction solutions induced desorption of additional inner-  
398 spherical bound Cd from organic matter and hydroxides, and led to a pool-size effect  
399 (Wiederhold, 2015) by shifting the isotopic composition of the dissolved Cd towards the  
400 isotopic composition of the soil Cd. This effect was also reflected in the higher Cd  
401 concentrations in the extracts compared to the natural soil solution (Table S1 and S2).

402

#### 403 *Cd Uptake by Plants*

404 Plants acidify rhizosphere soils to mobilize nutrients (Clemens et al., 2002; Welch, 1995) and  
405 there is evidence that the wheat and barley plants studied here did accordingly with the side  
406 effect of increasing Cd availability. First, Cd concentrations of wheat and barley were related  
407 to the Cd concentrations of the soils (Figure S4) rather than the Cd concentrations of the soil  
408 solutions. This suggests that the wheat and barley plants created microenvironments in the  
409 rhizosphere soil where pH values were lower and more homogeneous among the sites than  
410 they were for the bulk soils. As a consequence, the dissolved Cd concentrations of these  
411 microenvironments were mainly determined by the bulk soil Cd concentrations (Christensen,  
412 1984). Second, the measured Cd stock of the wheat and barley biomass from OE and NE was  
413 two orders of magnitude higher than the expected value (Figure S5). A different result was  
414 obtained for WI, the site with the lowest pH value, where the measured Cd stock of wheat  
415 and barley was similar to the expected value. The large offset observed at OE and NE might  
416 be due to the alteration of the rhizosphere soils by the wheat and barley plants and enhanced  
417 Cd availability, for example by soil pH reduction through release of organic acids and protons

418 (Collins et al., 2003; Naidu and Harter, 1998). Notably, Visual MINTEQ calculations revealed  
419 that both, decreasing solution pH and increasing solution Cd concentrations, enhance the  
420 share of free Cd<sup>2+</sup> in soil solutions. As free Cd<sup>2+</sup> is generally thought to be the main Cd form  
421 that is taken up by plants (Mendoza-Cózatl et al., 2011), the acidification of the rhizosphere  
422 might have increased Cd<sup>2+</sup> uptake by plants. The latter is facilitated by membrane proteins in  
423 phytoplankton and plants which transport nutrients as divalent cations, such as Fe<sup>2+</sup>, Zn<sup>2+</sup> and  
424 Mn<sup>2+</sup> (Horner et al., 2013; Page and Feller, 2015; Yamaji and Ma, 2014). Because the targeted  
425 nutrients are chemically similar to Cd, plants also take up the non-essential Cd (Khan et al.,  
426 2014). In contrast, the exudation of phytosiderophores by wheat plants grown on metal-  
427 enriched soils, have not been observed to mobilize Cd unlike Cu, Ni and Zn (Puschenreiter et  
428 al., 2017).

429 The subsequent preliminary model (Figure 3) describes five processes which together can  
430 explain the Cd isotope compositions of whole wheat and barley plants.

431 (#1) *Cd desorption from soils* - desorbed Cd is isotopically heavier than adsorbed Cd: The  
432 differences in Cd isotopic composition between solid soils and whole wheat and  
433 barley plants could be caused by the isotopic fractionation between the solid and  
434 solution phases and the amount of desorbed Cd. Mobilization of Cd is furthermore  
435 passively enhanced by plants. Consequently, the isotopic signature of the dissolved  
436 Cd, that is taken up by plants, shifts towards Cd isotope compositions of the soils  
437 and is lighter than the undisturbed soil solutions but still heavier than the bulk soils.  
438 This pool-size effect is also revealed by the relationship between the plant Cd  
439 stocks and  $\Delta^{114/110}\text{Cd}_{\text{soil-plant}}$  values (Figure 4). At OE and NE, the wheat plants  
440 contained four and two times more Cd than barley plants, respectively. This might  
441 reflect that wheat plants are more efficient in mobilizing Cd than barley plants and  
442 hence the former are isotopically lighter than the latter because of the stronger  
443 pool-size effect. This phenomenon could be attributed to the same pool-size effect  
444 as observed with the Cd extracts and the soil solution, where the smaller mobilized  
445 fraction (soil solution and barley) showed the heavier values, while mobilization of  
446 more Cd (extracts and wheat) caused the isotope ratios to become lighter. At WI in  
447 contrast, wheat and barley plants have about the same Cd stock, in accord with the  
448 observation that both plants have similar isotopic compositions.

449 (#2) *Cd speciation in soil solutions* - free Cd<sup>2+</sup> is isotopically heavier than Cd associated  
450 with DOM: The isotope fractionation between different dissolved Cd species is  
451 hitherto unstudied. However, Cd bound to soil surfaces is known to be isotopically  
452 lighter than dissolved Cd (Figure 2, Wasylenki et al., 2014; Zhang et al., 2016) and  
453 organic matter is considered to be the most important Cd sorbent in soils. Thus,  
454 from that theoretical point of view, we would expect the free Cd<sup>2+</sup> – which is the  
455 main form taken up by plants – to be isotopically heavier than DOM-bound Cd  
456 (Clemens and Ma, 2016; Perfus-Barbeoch et al., 2002). This expectation is in line  
457 with the shorter and stiffer bonds of hydrated Cd (Ohtaki and Johansson, 1981;  
458 Vasconcelos et al., 2008) than of Cd bound to the S ligands of organic matter  
459 (Karlsson et al., 2005). At our sites, the Cd speciation differed between the sites,  
460 with the predominant part of the dissolved Cd being present as free Cd<sup>2+</sup> at WI and  
461 as DOM bound Cd at OE and WI. Despite these differences, the isotopic  
462 fractionation between the soil and soil solution was constant among the sites. This  
463 is a hint that Cd speciation in solution did not influence the Cd desorption from the  
464 soil.

465 (#3) *Cd adsorption on root surfaces* - adsorbed Cd is isotopically lighter than dissolved  
466 Cd: It is well known that the negatively charged root surfaces and the apoplast of  
467 plants grown in soils or hydroponics are important sorption sites for Cd<sup>2+</sup>  
468 (Sattelmacher, 2001; Uraguchi et al., 2009). When the mechanisms described  
469 above come into play, the adsorbed Cd will be isotopically lighter than the soil  
470 solution Cd (Horner et al., 2011; Wasylenki et al., 2014; Zhang et al., 2016).

471 (#4) *Cd diffusion* - faster movement of light isotopes: It was previously demonstrated  
472 that free ions and lighter isotopes diffuse faster than complexed ions and heavier  
473 isotopes (Rodushkin et al., 2004). Diffusion therefore supports the preferential  
474 movement of light Cd isotopes from soil solutions to root surfaces and from the  
475 apoplast solution to metal transporters (Jouvin et al., 2012).

476 (#5) *Plant uptake of Cd* - with either no isotope fractionation or a slight enrichment of  
477 light Cd isotopes: To enter the plant, Cd<sup>2+</sup> ions generally need to cross a plasma  
478 membrane via ion channels or transporters for essential nutrient metals (Clemens  
479 and Ma, 2016; Perfus-Barbeoch et al., 2002). Nonspecific Cd uptake by

480 phytoplankton is not associated with isotopic fractionation because of fast  
481 equilibration or non-fractionating exchange with the surrounding medium (Horner  
482 et al., 2013). In contrast, Cd-tolerant hyperaccumulator plants, grown in  
483 hydroponics, were enriched in light Cd isotopes relative to the nutrient solutions  
484 (Wei et al., 2016). Cadmium uptake could occur via metal transporters or ion  
485 channels that may impart covalent binding (Nelson and Cox, 2005). Hence, Cd  
486 uptake is expected to produce either an enrichment of light isotopes in plants, due  
487 to the Cd bonding with the transporter (Horner et al., 2011; Wasylenki et al., 2014;  
488 Zhang et al., 2016) or show no isotopic fractionation.

489 Overall, wheat and barley plants were observed to be isotopically heavier than soils, and this  
490 demonstrates that processes #1 and #2 were more important for the isotope fractionation  
491 between solid soils and plants than processes #3, #4, and #5. This finding is further confirmed  
492 with the positive correlation between the  $\Delta^{114/110}\text{Cd}_{\text{soil-plant}}$  values (Figure 4) and the Cd stocks  
493 in plants. The higher the Cd stock in plants was, the isotopically lighter got the Cd in plants.  
494 This indicates that a pool-size effect after Cd desorption (#1) enhanced free  $\text{Cd}^{2+}$   
495 concentrations at lower pH (#2) determined the apparent isotopic fractionation between soils  
496 and plants. In contrast, pool-size effects during Cd adsorption on root surfaces (#3), Cd  
497 diffusion (#4), or plant uptake of Cd (#5) would lead to isotopically heavier Cd in plants.

498

#### 499 *Cd Redistribution in Plants*

500 Cd concentrations decrease from roots to straw and grains for both plants and all three sites  
501 (Figure S3) which is in line with previous observations (Page and Feller, 2015). The plants  
502 hence hindered transport of toxic Cd into the growing plant tissues. Such detoxification  
503 strategies are used by various organisms and might be similar between bacterial and human  
504 cells (Prévéral et al., 2009). In general, it is assumed that Cd is bound to phytochelatin or  
505 glutathione in the cytosol and excreted or sequestered afterwards, e.g. into vacuoles or cell  
506 walls (process #6, Figure 3, Clemens et al., 2002; Prévéral et al., 2009). This sequestration also  
507 causes isotopic effects with Cd isotope signatures that become heavier from roots to straw  
508 and grains. This observation is in line with results for wheat plants grown in a pot study on  
509 soils from the same locations (Wiggenhauser et al., 2016). Theoretical calculations showed

510 that sulfides are more enriched in light Cd isotopes than hydrated Cd (Yang et al. 2015). The  
511 same isotope behavior was described for Hg which forms very strong Hg-S bonds, with the  
512 lighter isotopes enriched in the bonding due to soft-acid soft-base interactions and lower  
513 vibrational frequencies (Wiederhold, 2015). Consequently, the sequestered or excreted Cd (in  
514 roots and straw, process #6, Figure 3) is isotopically lighter than the transported Cd (process  
515 #7). Therefore, the new, emerging plant tissues show lower Cd concentrations and are  
516 enriched in heavy Cd isotopes.

517 The Cd distribution and isotope fractionation in wheat can be described with a closed system  
518 Rayleigh fractionation model (Figure 5, Wiederhold, 2015; Wiggerhauser et al., 2016). A pot  
519 study thereby revealed different isotope fractionation factors for Cd retention in roots  
520 compared to shoots, in roots and straw compared to grains, and in straw compared to grains  
521 (grey lines in Figure 5, Wiggerhauser et al., 2016). The model assumes that the extent of the  
522 isotope fractionation depends on the amount of Cd that is translocated from one plant part to  
523 the next. To compare controlled conditions with field conditions, our new data for plants  
524 grown at OE, WI and NE and the pot study results are both shown in Figure 5. Notably, the  
525 Rayleigh fractionation lines that were fitted for the combined wheat data of both studies  
526 (colored lines in Figure 5), show only marginal differences to the best fit lines for the pot-  
527 study data only. Hence, the isotopic fractionation does not vary strongly between greenhouse  
528 and field, different soil types and different wheat cultivars – for all three described  
529 fractionation processes.

530 However, the apparent isotopic fractionation between roots-straw and grains (Figure 5b) as  
531 well as between straw and grains (Figure 5c) differed between the two crops, although the  
532 mass fractions of Cd transported into grains were in the same range for barley and wheat (11  
533 to 44%, Figures 5b and 5c). Thus, the processes involved in grain filling might differ between  
534 the wheat and barley cultivars chosen in this study. Isotope labeling studies showed that Cd is  
535 directly transported from root to grain and remobilized from stem and leaves during grain  
536 filling of wheat (Riesen and Feller, 2005; Yan et al., 2018; Harris and Taylor, 2001). The main  
537 processes that determine remobilization and metal isotope fractionation might be adsorption  
538 in the apoplastic space, complexation and compartmentalization in the cells, phloem mobility,  
539 and membrane transport (Wiggerhauser et al. 2018). The distinct apparent isotope  
540 fractionation suggests that the contribution of the processes that control remobilization

541 differed between wheat and barley. For instance, Cd retention in straw induced by  
542 complexation to thiol ligands is expected to cause a stronger retention of light isotopes  
543 compared to binding to carboxylates (apoplastic binding sites) if bond lengths not only control  
544 the direction but also the extent of isotope fractionation (see section “Cd in soil and soil  
545 solution”). However, more experimental data is needed to strengthen such isotope process  
546 tracing approaches for Cd. Furthermore, the contributions from remobilized Cd to grain Cd  
547 differs among wheat cultivars (Harris and Taylor, 2001) which suggests the differences found  
548 in this study could be also caused by the wheat and barley cultivars and not only by the  
549 different plants species. We only found one study that directly compared (net) remobilization  
550 patterns of wheat and barley (Maillard et al. 2015). It revealed that the net remobilization of  
551 most micronutrients is similar in wheat and barley when compared to e.g. maize, rape, and  
552 pisum sativum. To our knowledge, no such comparison exists for Cd. Together, our isotope  
553 analysis showed that processes that determine the Cd transport into the grains can differ  
554 between wheat and barley.

555

## 556 Conclusion

557 i) The isotope fractionation between soil and soil solution was not affected by any other  
558 property of the soil solid phase or soil solution. The constant isotopic fractionation  
559 between the sites suggests that Cd was primarily bound to organic matter and  
560 hydroxides as inner-sphere complex. The heavier isotope compositions in the soil  
561 solution compared to soil extracts indicate a pool-size effect controlling the Cd  
562 isotope composition of the Cd in soil solution.

563 ii) Desorption from soil, soil solution speciation, adsorption on root surfaces, diffusion,  
564 and plant uptake were identified as the responsible processes for the Cd isotope  
565 fractionation between soil, soil solution and plants. Thereby, the first two process  
566 dominated over the other processes. This valuable finding can be used in future  
567 research in which Cd stable isotopes are involved.

568 iii) Within plants, compartments with lower Cd concentrations were enriched in light  
569 isotopes, following a Rayleigh fractionation model. The fractionation and retention  
570 might be a consequence of Cd retention mechanisms, cereals use to prevent the  
571 transport of the toxic Cd to the grain. Barley cultivars were more efficient in Cd  
572 retention, showing lower Cd concentrations and stronger isotope fractionation,  
573 than wheat cultivars.

574

## 575 Supplementary Material

576 Section 1: Material and methods (detailed information). Figure S1: Experimental sites. Figure  
577 S2: Selected soil properties. Figure S3: Plant properties. Figure S4: Relationship between soil  
578 and plant Cd concentrations. Figure S5: Comparison of expected with measured Cd stocks in  
579 wheat and barley. Table S1: Soil solution properties. Table S2: Soil properties. Table S3:  
580 Standard reference materials.

581

## 582 **Acknowledgments**

583 This study was funded by the Swiss Parliament via the National Research Program (NRP) 69  
584 “Healthy Nutrition and Sustainable Food Production” (SNSF grant no. 406940\_145195/1). We  
585 thank the farmers from the study sites for cooperation, Lorenz Schwab for the  
586 characterization of the soils, Carl Palk and Rebekah Moore for the support with data  
587 acquisition, Barry Coles for the help in the MAGIC laboratories and the plant nutrition group  
588 for the guidance through the plant digestion procedures. Many thanks to the members of the  
589 soil science and TrES group at the University of Bern for support in the laboratory and helpful  
590 discussions.

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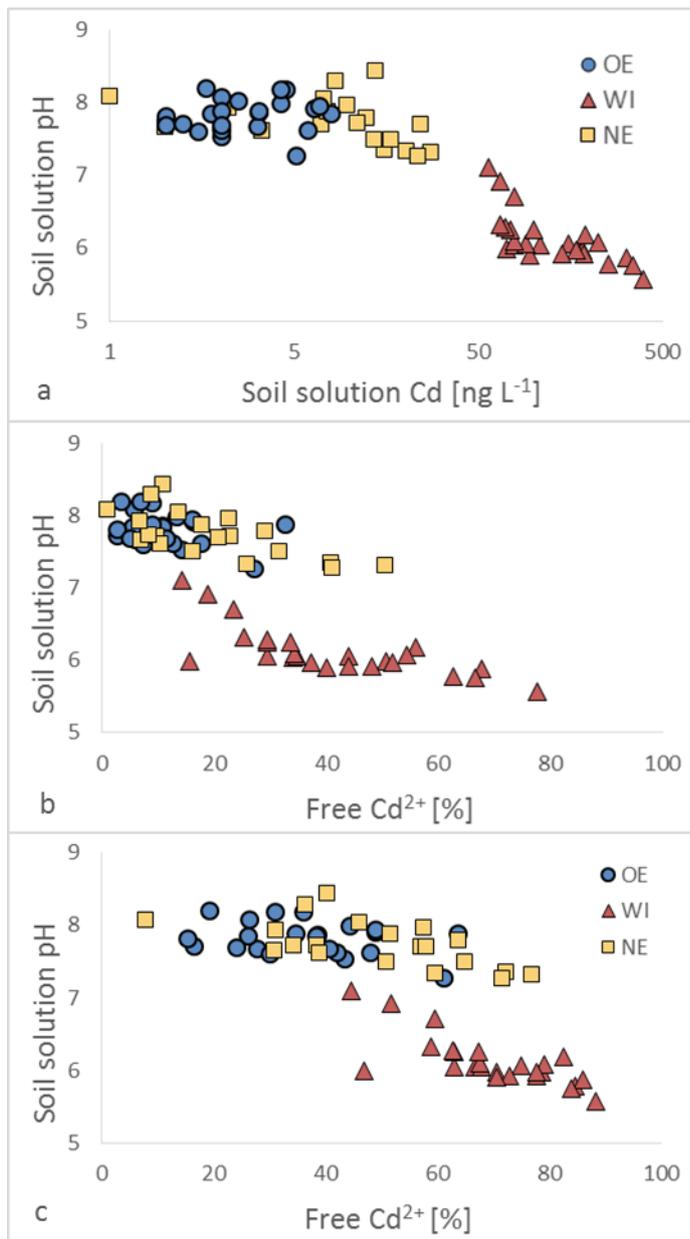
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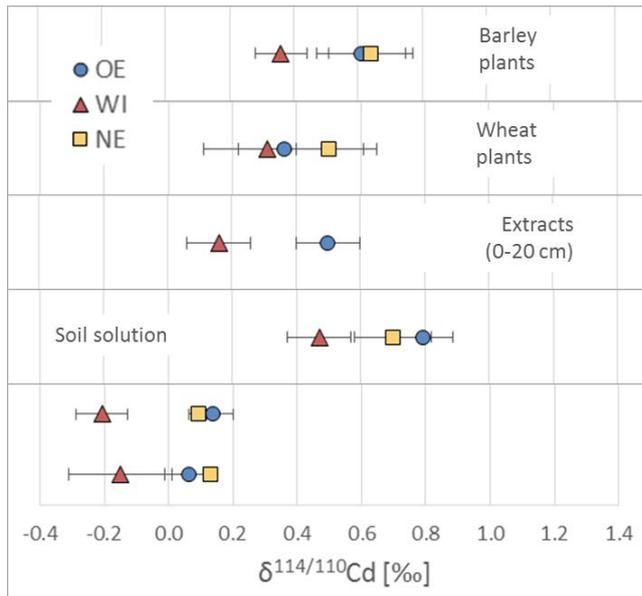
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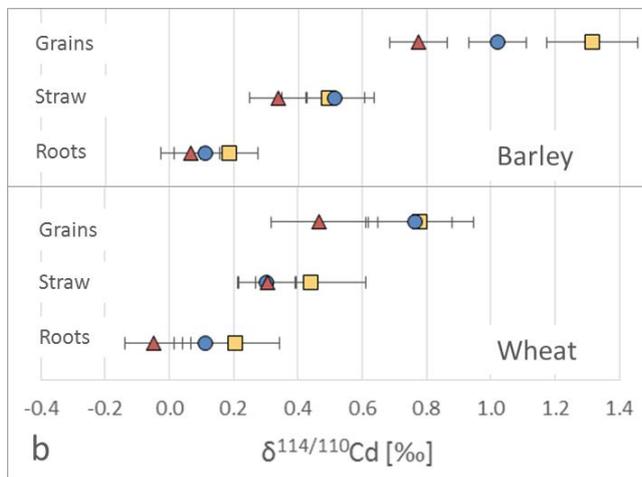
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881 Figure 1. Relationship between measured soil solution pH and (a) measured soil solution Cd  
 882 concentrations, (b) modelled fraction of free Cd<sup>2+</sup> in soil solutions with the max-HS scenario,  
 883 and (c) modelled fraction of free Cd<sup>2+</sup> in soil solutions with the min-HS scenario. Max-HS and  
 884 min-HS refer to the two model scenarios with maximum (60%) and minimum (20%)  
 885 contributions of humic substances to total DOM concentrations. Each data point represents a  
 886 biweekly measured or modelled value.

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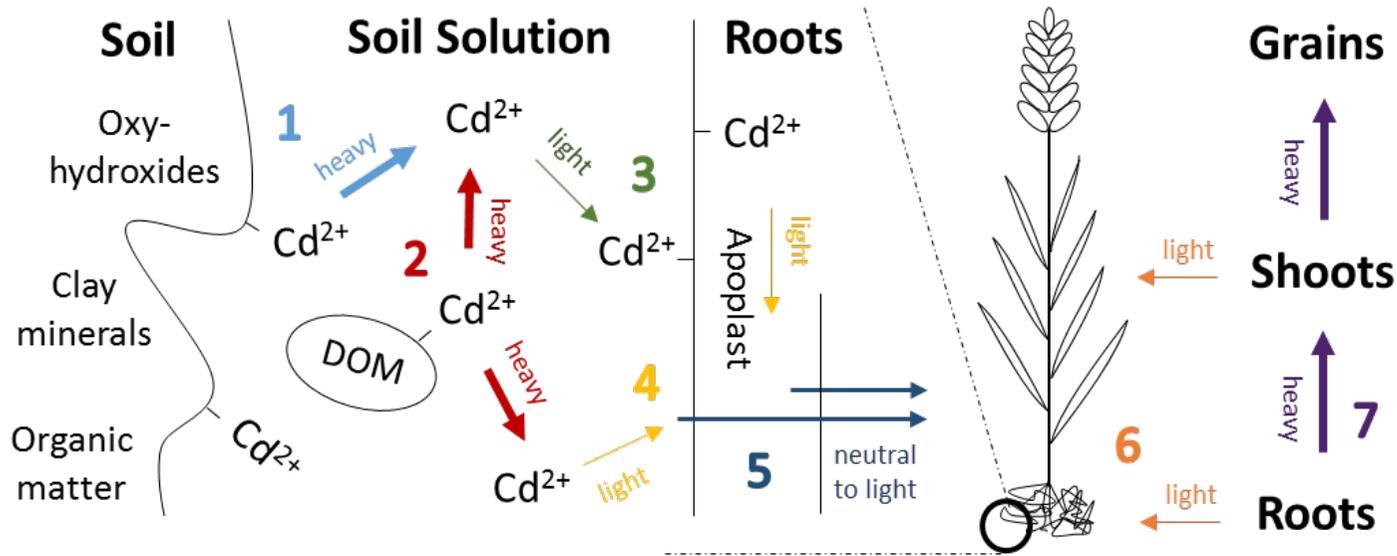
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890 Figure 2. Cd isotope compositions of (a) whole barley and wheat plants, 0.05 M Ca(NO<sub>3</sub>)<sub>2</sub>  
 891 extracts from soils (Wiggenhauser et al., 2016), soil solutions and soils (Imseng et al., 2018)  
 892 and (b) grains, straw, and roots of barley and wheat plants. Error bars represent 2 x standard  
 893 deviations of sample replicates where n>1 and measurement replicates where n=1. Minimal  
 894 2SD was the external reproducibility of 0.09‰. The isotope compositions of whole wheat and  
 895 barley plants were calculated according to Equation 3, with error propagation according to  
 896 Kusonwiriawong et al. (2017).

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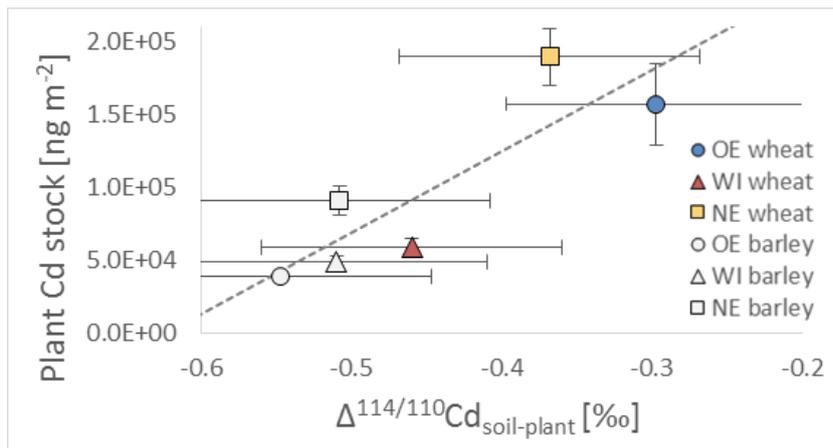


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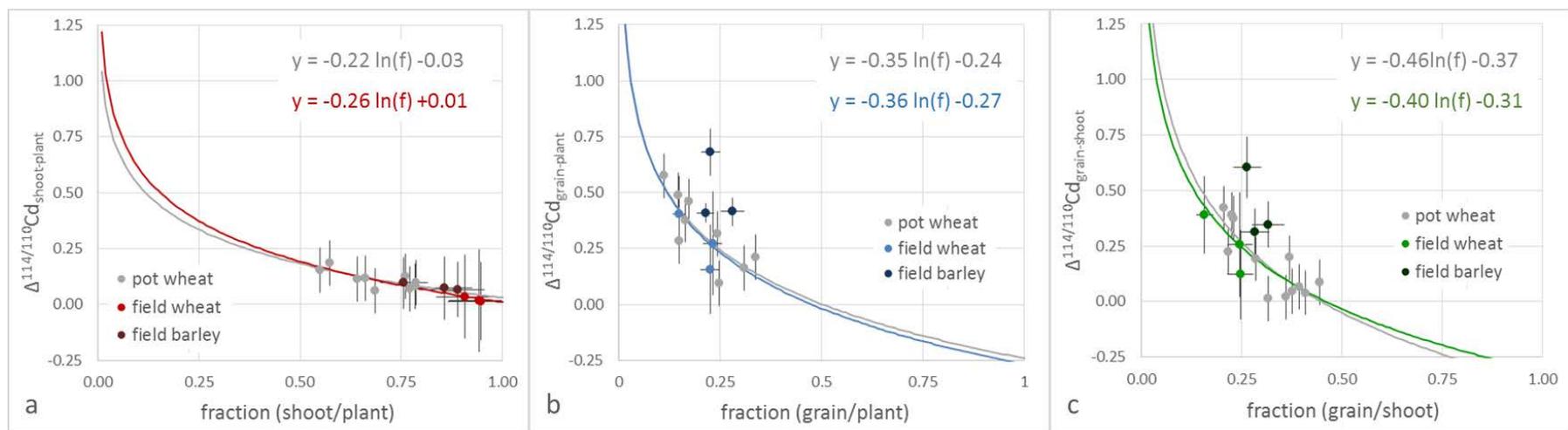
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901 Figure 3. Suggested mechanisms for the Cd isotopic fractionation between soils, soil solutions, and plants and within plants. The thickness of the  
902 arrows illustrates the direction of the isotopic fractionation of the five processes in the rhizosphere (#1) Cd desorption from soils, (#2) Cd speciation  
903 in soil solutions, (#3) Cd adsorption on root surfaces, (#4) Cd diffusion, and (#5) plant uptake of Cd as well as the plant-internal redistribution  
904 processes Cd sequestration (#6) and Cd transport (#7).



905

906 Figure 4. Relationship between the plant Cd stocks of wheat and barley and  $\Delta^{114/110}\text{Cd}_{\text{soil-plant}}$   
 907 values. Error bars represent standard deviations of sample replicates where  $n > 1$  and  
 908 measurement replicates where  $n = 1$  and were calculated with error propagation according to  
 909 Kusonwiriwong et al. (2017).



910

911 Figure 5. Rayleigh fractionation models for the field-grown wheat and barley plants of this study and wheat plants of a previous pot study. The best-  
 912 fit fractionation trends for the wheat of the pot experiment are shown as grey lines (Wiggenhauser et al., 2016), whereas the colored lines refer to  
 913 the best-fit fractionation trends for the wheat of pot and field experiments. (a) Cd retention in roots compared to shoots (straw and grains), (b) in  
 914 roots and straw compared to grains, and (c) in straw compared to grains. Error bars represent 1 x standard deviation for the mass fractions and 2 x  
 915 standard deviation for  $\Delta^{114/110}\text{Cd}$  values. Error propagation was calculated according to the equations from Kusonwiriya Wong et al. (2017).