Cadmium isotope fractionation in soil-wheat systems

Revised version June 2016

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Abstract

Analyses of stable metal isotope ratios constitute a novel tool to improve understanding of biogeochemical processes in soil-plant systems. In this study, we used such measurements to assess Cd uptake and transport in wheat grown on three agricultural soils under controlled conditions. Isotope ratios of Cd were determined in the bulk C and A horizons, in the $Ca(NO_3)_2$ extractable Cd soil pool and in roots, straw and grains. The $Ca(NO_3)_2$ extractable Cd was isotopically heavier than the Cd in the bulk A horizon ($\Delta^{114/110}Cd_{extract\text{-}Ahorizon}$ = 0.16 to 0.45‰). The wheat plants were slightly enriched in light isotopes relative to the $Ca(NO_3)_2$ extractable Cd or showed no significant difference ($\Delta^{114/110}Cd_{wheat\text{-}extract}$ = -0.21 to 0.03‰). Among the plant parts, Cd isotopes were markedly fractionated: straw was isotopically heavier than roots ($\Delta^{114/110}Cd_{straw\text{-}root}$ = 0.21 to 0.41‰) and grains were heavier than straw ($\Delta^{114/110}Cd_{grain\text{-}straw}$ = 0.10 to 0.51‰). We suggest that the enrichment of heavy isotopes in the wheat grains was caused by mechanisms avoiding accumulation of Cd in grains, such as the chelation of light Cd isotopes by thiol-containing peptides in roots and straw. These results demonstrate that Cd isotopes are significantly and systematically fractionated in soil-wheat systems and the fractionation patterns provide information on biogeochemical processes in these systems.

Introduction

Cadmium is a hazardous trace element for humans. As Cd has a long biological half life, small but continuous intake can lead to accumulation of Cd in the human body, causing reduced kidney function or weakening of bones.[1] Cadmium enters agro-ecosystems via mineral phosphate fertilizers, leading to an increase in plant-available Cd in agricultural soils.[2, 3] Although Cd is not a plant nutrient, plants take up small amounts of the metal, which then enters the food chain.

Improvements in analytical methods and instrumentation over the last two decades have enabled previously intractable measurements of minor variations in the stable isotope compositions of elements heavier than 40 amu.[4, 5] More recently, several studies conducted isotopic analyses of micronutrients, including Zn[6-8], Cu[7, 9], Fe[10, 11] and Ni[12], in soils and plants and showed that such data can provide valuable insights into the uptake and internal redistribution processes of these metals within plants. In principle, similar isotopic studies to investigate Cd uptake and transport by plants are also feasible, based on the available evidence.

First, the transfer of Cd from soils into plants is associated with numerous processes that are conducive to the generation of stable isotope fractionation. For example, the sorption of Cd to soil surfaces and organic compounds is strongly influenced by soil pH, which determines the plant availability of Cd.[13, 14] Cadmium is also known to sorb to the negatively charged surfaces of the root apoplast[15-17] where it can also precipitate with phosphates.[18] In the soil solution and in several plant parts, Cd is furthermore complexed by inorganic and organic ligands.[13, 18-24] As Cd is non-essential and toxic at comparatively low concentrations

61 to most plants,[13, 25] the metal is also typically retained in roots and straw, which produces
62 decreasing Cd concentrations in the order root > straw > grain.[20, 26]

63 Second, a number of individual studies have already revealed significant Cd isotope
64 fractionations in environmental samples. Lab experiments, for example, showed that lighter
65 Cd isotopes are preferentially adsorbed to Mn-oxyhydroxides[27] and enriched in calcite
66 during the precipitation of this phase from aqueous solutions.[28] The membranes of
67 *Escherichia coli* were also found to be enriched in light Cd isotopes, as a result of Cd
68 sequestration onto membrane thiols.[29] Recently published studies furthermore determined
69 Cd isotope compositions for plants that differed significantly from the natural geological
70 background.[30-32] Cadmium-tolerant plants grown in polluted environments were thereby
71 found to have an enrichment of isotopically light Cd,[31] whilst birch leaves from industrial
72 areas showed heavy Cd isotope compositions.[30]

73 Here, we present the results for the first comprehensive and systematic study of Cd isotope
74 variations for soil-plant systems at non-contaminated conditions. To this end, wheat was
75 cultivated under controlled conditions on three different arable soils. At full maturity,
76 samples were taken from different plant parts and analyzed for Cd concentrations and
77 isotope compositions. With additional analyses of soil samples, we were able to fully
78 characterize the mass balance, fluxes and isotopic fractionation of Cd between soils and
79 plants, between different soil pools and between different plant parts. Evaluation of these
80 results in the context of isotope mass balance models enabled us to systematically discuss
81 the processes responsible for the observed fractionations and relate these to the retention
82 mechanisms, which help wheat to avoid excessive Cd accumulation in grains.

83

Materials and Methods

Plant growth experiment

The soils were sampled from the A (0-20 cm) and C horizons of three study sites of the Swiss Soil Monitoring Network (NABO[33]). The soils were considered representative for Swiss wheat production and originated from Oensingen (Oen), Landquart (LQ) and Wiedlisbach (Wied).[33] These soils differ in properties that determine the plant availability[13] of Cd, such as total Cd abundance, pH, C concentration and texture (Table S2, NABO[33]). Soil samples were air-dried and sieved to < 5 mm before being used for the growth experiment.

Plants were cultivated in pots filled with 1 kg of dry soil from the A horizon. For each soil, four replicates were prepared. To ensure regular plant growth, macro- and micronutrients were added. Wheat seeds of *Triticum aestivum* L., cv. "Fiorina" were sterilized using 10% H_2O_2 and sown. The pots were then placed in a growth chamber and sufficiently watered with deionized water (>18.2 MΩ). Further details are described in the Supporting Information (section 1.1).

Sample preparation

Roots, straw (stem and leaves) and grains were harvested at full maturity. After shoots (straw and grains) were collected and separated, the roots were carefully separated from the soil and washed with 6 mM $NaNO_3$.[34] Plant samples were dried at 55°C and homogenized in a rotary mill equipped with tungsten carbide cups. A high pressure single reaction chamber microwave system was used to digest the plant samples with HNO_3. The A and C horizons were digested using a mixture of HNO_3 and HF in a microwave oven. Soil pools considered as plant available Cd were extracted from the A horizons using 0.05 M $Ca(NO_3)_2$[35]

followed by microwave digestion to destroy extracted organic complexes. Further details are described in Supporting Information (section 1.2.).

Analyses and standard reference materials

Aliquots of each soil and plant sample were taken to determine the concentrations of Cd and macro- and micronutrients. The Cd concentrations for each of the four replicate plant samples that were grown in separate pots were determined by quadrupole ICP-MS. Three out of the four individual replicates were randomly chosen and individually digested and purified for isotope analyses. The Cd isotope compositions of soils were measured on two individually digested and purified samples for each A and C horizons. The stable Cd isotope compositions of all samples were thereby determined using a double spike technique and multiple collector inductively coupled plasma mass spectrometry (MC-ICP-MS).[36, 37] Procedural blanks were processed alongside samples during digestion (n=9) and the matrix separation required prior to the isotopic analyses (n=10), to monitor the impact of laboratory-induced contamination. The total procedural Cd blank for the isotopic measurements ranged from about 140 to 530 pg. This is equivalent to less than 1% of the indigenous Cd for the smallest samples, such that blank corrections were not required for the isotopic data. A number of standard reference materials (SRMs) were analyzed alongside samples for quality control and provided robust results, as documented in the Supporting Information (section 1.2 and Table S1).

Calculations and statistics

The isotope compositions of the samples are reported relative to the NIST 3108 Cd isotope reference material[38] using a δ notation based on $^{114}Cd/^{110}Cd$:

$$\delta^{114/110}\mathrm{Cd} = \left[\frac{(^{114}\mathrm{Cd}/^{110}\mathrm{Cd})_{\mathrm{sample}}}{(^{114}\mathrm{Cd}/^{110}\mathrm{Cd})_{\mathrm{NIST3108}}} - 1\right] \times 1000 \qquad (1)$$

The $\delta^{114/110}Cd$ values for whole plant or shoot (straw + grain) samples were calculated as:

$$\delta^{114/110}\mathrm{Cd}_{\text{whole plant or shoot}} = \frac{\sum_i m_i c_i \delta^{114/110}\mathrm{Cd}_i}{\sum m_i c_i} \qquad (2)$$

where m represents the mass of dry matter (DM, in g), c the Cd concentration (ng g^{-1}), and i the different plant parts of the whole plant (root + straw + grain) or of the shoot (straw + grain).

The apparent isotopic fractionation between soil pools and/or plant parts was calculated as:

$$\Delta^{114/110}\mathrm{Cd}_{A-B} = \delta^{114/110}\mathrm{Cd}_A - \delta^{114/110}\mathrm{Cd}_B \qquad (3)$$

where A and B denote the soil pools or the plant parts of interest in the soil-wheat system (e.g. root, straw, whole-plant, $Ca(NO_3)_2$ extractable Cd).

For wheat samples, where 3 or 4 individual replicates were available, significant differences in DM, Cd concentration, Cd uptake, and $\delta^{114/110}Cd$ were determined using ANOVA followed by a Tukey HSD test. The level of significance was set to $p < 0.05$. However, soil samples were analysed only in analytical duplicate. In this case, the results for the two samples were (conservatively) considered to be different, if the mean values showed no overlap within the combined 2sd uncertainties of the data. The quoted uncertainties (Table S1) were obtained from the ±2sd reproducibility that was determined from replicate analyses for relevant SRMs

of both geological (AGV-2, NIST 2709a; n = 3) and biological (NIST 1567b; n = 6) character. These replicate analyses, with separate dissolutions and sample processing, yielded $\delta^{114/110}$Cd values with ±2sd precisions of ±0.065‰ and ±0.083‰ for the geological and biological SRMs, respectively. All statistical tests and correlations were computed with the statistical software R (version 3.1.3).

Results

Soil properties

The studied soils varied in pH (5.2 – 7.1), texture, cation exchange capacitiy (CEC, 44-217 $mmol_c\ kg^{-1}$) and C concentration (15 to 57 g kg^{-1}, Table S2). Total Cd concentrations in the A horizons ranged from 193 to 508 ng g^{-1} and were 1.7 to 3.4 times higher than in the C horizon. Such soil Cd concentrations can be considered typical for non-contaminated environments.[13] The $Ca(NO_3)_2$ extractable Cd was higher in the acidic soils (Oen: 20 ng g^{-1}, Wied: 40 ng g^{-1}) than in the soil with neutral pH (LQ: 3 ng g^{-1}). The contribution of $Ca(NO3)_2$ extractable Cd to total Cd pool of the A horizon was largest for Wied (21%), and much smaller in Oen (4%) and LQ (1%).

Plant growth and Cd distribution among the different plant parts

Dry matter production for the different plant parts grown on the same soil decreased in the order grain > straw > root (Figure S1a, Table S3). Comparing the same plant parts grown on different soils revealed that DM production of straw in Wied was significantly higher than in Oen. Cadmium concentrations were highest in the roots (296 to 351 ng g^{-1}), followed by straw (100 to 142 ng g^{-1}) and grains (26 to 63 ng g^{-1}, Figure S1b, Table S3). The Cd concentration of grains was closest to the upper limit for non-contaminated environments (100 ng g^{-1})[13] in Oen (63 ng g^{-1}) whilst grains from Wied and LQ had much lower Cd contents. Most Cd was accumulated in the straw (541 to 917 ng plant $part^{-1}$) followed by the roots (258 to 668 ng plant $part^{-1}$) and grains (174 to 397 ng plant $part^{-1}$, Figure S1c). In comparison to other micronutrients such as Zn or Cu, Cd was retained in root and straw, which contained on average 29% and 51%, respectively, of the total Cd present in the wheat plants. Hence,

only 20% of the Cd in wheat reached the grains, whereas 69% of Zn and 62% of Cu was present in grains (Figure S2).

Cd isotope fractionation in soils and wheat

The Cd isotope data for the A and C horizons showed little variation (Figure 1, Table S4) whilst significant differences are apparent between the bulk A horizon (Ahor) and $Ca(NO_3)_2$ extracts for all soils. The mean $\Delta^{114/110}Cd_{extract\text{-}Ahor}$ values were thereby higher for Oen (0.43‰) and LQ (0.45‰) than for Wied (0.16‰).

Cadmium isotopes were also significantly fractionated among the different plant parts (Figure 1, Table S4). Cadmium in grains was isotopically heavier than Cd in straw and the latter was isotopically heavier than Cd in roots. The mean $\Delta^{114/110}Cd_{straw\text{-}root}$ values were highest for wheat grown in Oen (0.41‰) followed by LQ and Wied (0.21‰ to 0.24‰). In contrast, $\Delta^{114/110}Cd_{grain\text{-}straw}$ values were highest in Wied (0.50‰), followed by LQ (0.29‰) and Oen (0.10‰). The calculated Cd isotope compositions for the whole plants revealed that wheat was enriched in heavy isotopes compared to the bulk A horizon ($\Delta^{114/110}Cd_{wheat–Ahor}$ = 0.13 to 0.39‰, Table 1). However, the whole plants were identical, within the analytical uncertainty of ±0.08‰ (2 sd), to the $Ca(NO_3)_2$ extractable Cd for Oen ($\Delta^{114/110}Cd_{wheat–extract}$ = 0.03‰) and Wied (-0.03‰) but enriched in light isotopes for LQ (-0.21‰). The shoots were enriched in heavy isotopes compared to the bulk A horizon ($\Delta^{114/110}Cd_{shoot\text{-}Ahor}$ = 0.24 to 0.50‰) for all soils but unfractionated when compared with the $Ca(NO_3)_2$ extracts of the soils ($\Delta^{114/110}Cd_{shoot–extract}$ = -0.09 to 0.08‰).

Discussion

Cadmium isotope fractionation in soils

For all soils, the Cd of the A horizon was slightly enriched in heavy isotopes compared to the C horizon Cd, with $\Delta^{114/110}Cd_{Chor\text{-}Ahor}$ = -0.03 to -0.12‰ (Figure 1, Table S4). This could be a consequence of weathering processes or the application of Cd-rich P-fertilizers that feature isotopically heavy Cd. Until now, Cd isotope data for soils were only been obtained for contaminated environments, to trace anthropogenic inputs of Cd.[39, 40] These studies also revealed, however, that it can be difficult to distinguish between Cd isotope variations from anthropogenic activities and isotopic fractionations caused by weathering processes.[40] Furthermore, Lambelet et al.[41] found no or a slight enrichment of heavy isotopes in rivers compared to the continental crust and suggested that weathering processes have a minor impact on Cd isotope fractionation.

The difference in Cd isotope composition between the A and C horizons was largest for the Wied soils, which have the lowest pH, C concentrations and CEC (Table S2). If this difference is a consequence of weathering, this would indicate that Cd in Wied is more prone to loss by leaching, whilst Cd retention is more pronounced in LQ and particularly Oen where $\Delta^{114/110}Cd_{Chor\text{-}Ahor}$ is almost negligible. As the $Ca(NO_3)_2$ extracts have heavy Cd isotope compositions relative to the respective bulk soils, preferential loss of heavy Cd isotopes from soils is expected during weathering. This prediction, however, contradicts the observation that the weathered A horizons have more positive $\delta^{114/110}Cd$ values than the substrate rocks of the C horizon. Hence, it appears more likely that fertilizer-derived Cd is responsible for the shift towards heavy Cd isotope ratios in the A horizon. This would require, however, that the applied fertilizers were also characterized by heavy Cd isotope compositions. Regardless of

224 which processes are ultimately responsible for the shift towards heavy Cd isotopes in the A
225 horizon, the comparatively small fractionation between the A and C horizons (Figure 1, Table
226 S4) demonstrates that weathering processes and/or long-term Cd addition through P-
227 fertilizers had no major impact on the Cd isotopic composition of the bulk soils.

228 Our data reveal a clear apparent isotopic fractionation between the bulk soil and the
229 $Ca(NO_3)_2$ extractable Cd, which is considered to be the immediately plant-available Cd pool
230 of the A horizon (Figure 1, Table S4). This fractionation is similar in magnitude to the isotopic
231 difference observed between soil and soil solution in field experiments in Oen and Wied
232 ($\Delta^{114/110}Cd_{\text{soil solution-Ahor}} \approx 0.6‰$; own unpublished results). The isotopic composition of the
233 $Ca(NO_3)_2$ extractable Cd results from weathering processes and any soluble Cd that is added
234 to the soil from external sources. This Cd pool is weakly bound to the soil surfaces and the
235 sorption process itself might generate additional isotope fractionation, as reported by
236 Wasylenki et al.[27] These authors showed that birnessite preferentially adsorbs light Cd
237 isotopes and this produces an enrichment of heavy isotopes in the soluble Cd fraction
238 ($\Delta^{114/110}Cd_{\text{fluid-solid}}$ = 0.24 to 0.54‰). At equilibrium conditions, the heavier isotopes are
239 generally enriched in the phase with the stronger bonding environment, which is
240 determined by the number, length, and stiffness of the bonds.[27, 42, 43] In the context of our
241 study, this guideline suggests that the $Ca(NO_3)_2$ extractable Cd is more strongly bound in
242 complexes with dissolved ligands than to the soil sorption sites. In contrast to the purely
243 inorganic solutions of the birnessite sorption study,[27] the bulk A horizon and $Ca(NO_3)_2$
244 extracts analyzed here are likely to have contained organic ligands, and these are known to
245 produce stable isotope fractionations for many trace metals.[44-47]

246 The extent of the isotopic fractionation $\Delta^{114/110}Cd_{extract–Ahor}$ is related to the mass fraction of
247 Cd that is extractable using $Ca(NO_3)_2$ relative to the total Cd pool of the bulk A horizon
248 (Figure S3). Cadmium in the sandy loam soil of Wied was more exchangeable with $Ca(NO_3)_2$
249 than in the other soils, and exhibited the smallest apparent isotopic fractionation
250 $\Delta^{114/110}Cd_{extract–Ahor}$. The proportion of Cd that is extractable with $Ca(NO_3)_2$ relative to the
251 total Cd pool can affect the Cd isotope composition of the former because the extent of
252 equilibrium isotope fractionation is also determined by the fraction of bound versus
253 solubilized Cd.[27, 43] The size of the extractable Cd pool was expected to be influenced mainly
254 by the pH value,[13, 14] but our data revealed no significant difference in $\Delta^{114/110}Cd_{extract–Ahor}$
255 between Oen (pH = 5.6) and LQ (7.1) (Figure S3). This suggests that soil properties other than
256 pH, such as C concentration, CEC or texture, also had a strong impact on Cd sorption and
257 thereby the Cd isotope fractionation in soils. Experimental studies that investigated Cu[46, 47]
258 and Fe[45] isotope fractionation during complex formation demonstrate that stable organic
259 complexes are generally enriched in the heavy isotopes of these elements.[45, 47] It was also
260 shown that 50 to 70% percent of the Cd in soil solutions can be complexed by organic
261 ligands.[24] The presence of such ligands in the soil solution might hence affect Cd isotope
262 fractionation in a similar manner to that observed for other elements.

263

264 **Cadmium isotope fractionation between soils and wheat**

265 Figure 2 illustrates the apparent fractionation of Cd isotopes between selected soils pools
266 and plant parts of the soil-wheat system for Wied. The left column shows that the Cd isotope
267 composition of the whole plant was not fractionated relative to the $Ca(NO_3)_2$ extractable Cd.
268 Although the wheat plants were supplied with isotopically heavy $Ca(NO_3)_2$ extractable Cd
269 ($\delta^{114/110}Cd$ = 0.12‰), the roots were enriched in light isotopes with $\delta^{114/110}Cd$ = -0.12‰, as

shown in the central column. As 38% of the total Cd in the wheat plant was stored in the roots and enriched in light isotopes, the Cd exported to the shoot was isotopically heavier, with $\delta^{114/110}$Cd = 0.20‰, compared to the Cd pool in the root.

Similar observations were made for wheat from Oen but not for LQ (Figures S4-S5). In LQ, the Cd isotope composition of the whole plant was enriched in light isotopes relative to the $Ca(NO_3)_2$ extractable Cd. This may result from the complexation of Cd to organic ligands in the $Ca(NO_3)_2$ extract and in the bulk A horizon. Notably, such ligands are expected to be more abundant in the LQ soil as this exhibits a higher C content and pH value than the Wied and Oen soils (Table S2). In LQ, the $Ca(NO_3)_2$ extractable pool is only about three times larger than the total quantity of Cd in the plant (Figure S5). Considering these conditions, it is conceivable that only part of the $Ca(NO_3)_2$ extractable Cd pool of the LQ soil is plant-available in the form of free Cd^{2+} ions, whilst the remainder might be tightly bound to organic ligands.[13] If it is assumed that the ligand-bound Cd is isotopically heavy, in accord with observations for other trace elements,[45, 47] the plant-available Cd^{2+} ions from the extractable pool should isotopically light, with $\delta^{114/110}$Cd < 0.54‰. However, further tests are needed to confirm if heavy Cd isotopes are indeed preferentially bound to organic ligands in the A horizon and/or the $Ca(NO_3)_2$ extract. For soils with higher C concentrations than seen in LQ, it was already shown that O-, N- and S-containing ligands are important functional groups for binding Cd in soil and soil solution.[48, 49] Whilst metal complexes with organic ligands are typically enriched in heavy isotopes,[44-47] theoretical calculations suggest that S-containing ligands may preferentially complex light Cd isotopes to organic soil matter.[50] In particular, such modeling indicates that equilibrium conditions may favor the enrichment of isotopically light Cd in hydrosulfides relative to hydrate, hydroxide, chloride and nitrate forms of the element. This conclusion is supported by the observation of

294 isotopically light Hg bound to thiols relative to chloride and hydroxide forms of the
295 element.[51]

296

297 **Cadmium isotope fractionation in wheat**

298 The distribution of Cd within the different plant parts was associated with further Cd isotope
299 fractionation (Figure 2). In Wied, 80% of the Cd transported from the root into the shoot was
300 retained in the straw (right column of Figure 2). As this stored Cd was isotopically light with
301 $\delta^{114/110}$Cd = 0.09‰, an additional enrichment of heavy isotopes was observed for the grains
302 that were characterized by $\delta^{114/110}$Cd = 0.59‰.

303 The distribution of Cd amongst the plant parts (Figure S1c, S2) and the extent of the
304 associated isotope fractionation differed between the wheat plants grown on different soils
305 (Figure 1). Figure 3a shows that if the mass fraction of Cd stored in the shoot is larger, this
306 enhances the enrichment of light isotopes in the shoot. The same relationship was observed
307 for the Cd of the grains versus the Cd pool of the whole plants. Together, these results
308 demonstrate that the extent of Cd isotope fractionation between the various plant parts
309 depends on the extent of Cd translocation from the root into the shoot. The different plant
310 parts represent a flow-through system, whereby the transfer between and within the
311 individual plant parts and cells induce Cd isotope fractionation.[43] The successive use of the
312 plant-available Cd pool within the plant parts resembles closed-system dynamics[43] that
313 affect the redistribution of Cd isotopes in the plant. Closed-system dynamics can be
314 described with a Rayleigh mass-balance:[43, 52]

315 $$\Delta^{114/110}\mathrm{Cd}_{\mathrm{sink-source}} = \Delta^{114/110}\mathrm{Cd}_{\mathrm{retained-mobile}} \times \ln(\mathrm{f}) \quad (4)$$

316 The fractionation $\Delta^{114/110}Cd_{sink–source}$ is calculated with Equation 3, from the $\delta^{114/110}Cd$ values
317 of the sink and source, as given by the Cd isotope compositions of the whole plant and the
318 shoot, respectively. The apparent fractionation $\Delta^{114/110}Cd_{retained\text{-}mobile}$ is generated by the
319 retention of Cd in the roots.[52] Application of this model to the data reveals that storage of Cd
320 in the roots is associated with an isotope fractionation of $\Delta^{114/110}Cd_{retained\text{-}mobile}$ = -0.22‰
321 (Figure 3a). The same Rayleigh model can be also applied to the Cd pool stored in grains
322 (Figure 3a). In this case, $\delta^{114/110}Cd_{source}$ and $\delta^{114/110}Cd_{sink}$ of Equation 4 represent the isotope
323 composition of the whole plant and the grains, respectively. Consequently, $\Delta^{114/110}Cd_{retained\text{-}}$
324 $_{mobile}$ is the apparent fractionation that is produced by the retention of Cd in the roots and
325 straw and the modeling produces a result of $\Delta^{114/110}Cd_{retained\text{-}mobile}$ = -0.35‰ (Figure 4a).
326 Finally, Equation 4 can also be applied to the isotope fractionation of Cd between grains and
327 shoot (Figure 3b). In this scenario, $\delta^{114/110}Cd_{source}$ and $\delta^{114/110}Cd_{sink}$ are given by the isotope
328 composition of the shoot and the grain, respectively. Hence, $\Delta^{114/110}Cd_{retained\text{-}mobile}$
329 characterizes the apparent fractionation that is associated with Cd retention in the straw.
330 For these plant parts, the modeling produces a fractionation of $\Delta^{114/110}Cd_{retained\text{-}mobile}$ = -
331 0.46‰ (Figure 3b). The distinct $\Delta^{114/110}Cd_{retained\text{-}mobile}$ values that were obtained above
332 indicate that different processes might be responsible for the sequestration of Cd in the
333 straw and the roots.

334 We note that the results of the Rayleigh modeling must be treated with some caution. The
335 distribution of Cd in the roots, straw and grains was similar for all wheat plants.
336 Consequently, the mass fraction of Cd in the sinks shows only limited variation (Figure 3),
337 whereas a wider range would be desirable to improve the non-linear approximations. The
338 Rayleigh modeling, furthermore, assumes that there is no or only negligible transport of Cd
339 from the straw back into the roots[43] but such translocation has been identified by other

researchers.[53-55] However, Riesen and Feller[55] applied radiotracers to wheat leaves and the recovery of ^{109}Cd in the roots after 24 days was small in comparison to ^{63}Ni, ^{57}Co and ^{65}Zn. These results indicate that backward fluxes of Cd from the straw to the roots might be negligible and should have only a minor influence on the modelled Rayleigh fractionation curves.

In grasses such as rice, barley or wheat, Cd is thought to take a similar route from the soil solution into grains as the chemically similar element Zn.[56, 57] Both elements are, furthermore, not affected by redox processes, which are known to influence the isotope fractionation of elements such as Cu[58, 59] and Fe[60, 61] in plants. It is thus reasonable to compare the distribution and isotopic signature of Cd in plants with findings for Zn. Although both elements follow a similar pathway through grasses,[56, 57] Zn is more efficiently transported into wheat grains than Cd.[26, 53, 55, 62] In agreement with previous studies, Cd is retained primarily in roots and straw, whilst Zn accumulates more efficiently in grains (Figure S2). A comparison of the isotope fractionation for Cd in wheat and Zn in grasses reveals an inverse direction of the fractionation. The Zn in roots is isotopically heavier than that in the nutrient solution,[6, 59, 63] whilst the shoots and grains are isotopically lighter than the roots,[6, 59, 61, 63] and grains are isotopically lighter than the straw.[61] In contrast, the accumulation of Cd in roots and straw is associated with successively heavier Cd isotope compositions for the straw and grains. Together, these observations suggest that the Cd isotopic fractionation may be a consequence of mechanisms that work against the excessive accumulation of Cd in grains.

When Cd first enters the root apoplast, it can be adsorbed to the negatively charged surfaces of pectin-containing root cell walls.[16] Analogous to the Cd isotope fractionation that is

363 commonly associated with adsorption to mineral surfaces,[27] it is likely that Cd sorption to
364 root cell walls is also accompanied by a stable isotope effect. However, this sorption may
365 only play a subordinate role for Cd-sensitive plants, such as wheat, as compared to Cd-
366 tolerant plants.[64] In the root apoplast, Cd can precipitate as a phosphate[18] and such
367 reactions may also generate isotope fractionation, based on the observation that Cd
368 precipitation with calcite is associated with an enrichment of isotopically light Cd in the
369 carbonate.[28] The proportion of Cd that is precipitated by phosphate and the effect of this
370 process on Cd isotope compositions should hence be further investigated.

371 Facilitated by membrane transporters, Cd^{2+} is transported through the plasma membrane
372 into the root symplast where Cd can be further retained in the roots. In the cytoplasma, Cd^{2+}
373 can be transported through the tonoplast into the vacuole[65, 66] or it can be exported through
374 the plasma membrane into the apoplastic extracellular space.[67, 68] To be transported through
375 membranes, Cd binds to membrane proteins. Therefore, transport proteins could
376 preferentially bind light or heavy Cd isotopes in the same manner as they preferentially bind
377 specific metals.[66] Consequently, the transport of Cd^{2+} through the tonoplast and the export
378 through plasma membrane into the apoplast could contribute to a retention of light Cd
379 isotopes in the roots.

380 The chelation of Cd by thiol-containing peptides such as PC and/or further sequestration into
381 vacuoles has been identified as an important process of Cd retention in the roots of Cd-
382 sensitive plants.[69] In the cytosol, PCs are efficiently synthesized from glutathione by PC-
383 synthase in the presence of Cd[70] and Cd has a high affinity to thiols, especially to PC (K_d = 7.9
384 x 10^{-17}).[23, 71] Cadmium-PC complexes are less toxic than free Cd^{2+} [72] and can be further
385 transported into the vacuoles.[19, 66, 73] These processes have been also identified in wheat

where the chelation of Cd by thiols and sequestration of Cd-PC complexes can occur in environments with low Cd concentrations.[19, 62, 74] A recent Cd isotope study on *E. coli* furthermore revealed that the enrichment of isotopically light Cd in the cell membranes relative to the cytosol may reflect sequestration of Cd into membranes, where it is bound to cysteine-rich peptides.[29] The hypothesis that light Cd isotopes preferentially bind to thiols is further supported by theoretical calculations that evaluate Cd isotope fractionation in hydrothermal fluids[50] and, unlike elements such Cu, Zn and Fe, Hg bound to thiols was found to be isotopically light relative to chloride and hydroxide forms of the element.[51] Finally, vacuolar sequestration of Cd in the root cells would produce a successive fractionation of Cd isotopes, in accord with the Rayleigh fractionation trend defined by our data in Figure 3.

Once Cd and Zn are loaded into the xylem, both elements take a similar route into the grains. In the xylem, these metals move with the transpiration stream towards the leaves. Cadmium and Zn must then be loaded into the phloem to reach developing organs such as roots or grains.[56, 57] Phloem loading occurs in grasses in the nodes, stem and leaves and is facilitated by membrane transporters.[66, 75] Finally, Cd and Zn need to be unloaded from the phloem to be taken up by the seeds.[66, 75] To date, it is not known which transporters are involved in phloem unloading and whether Cd and Zn are transported into the grain in ionic or complexed form.[66, 75] Several processes that are inferred to produce Cd isotope fractionation in the root, including vacuolar sequestration of Cd-PC complexes, also occur in the straw.[19, 62, 66, 67] Therefore, the enrichment of heavy isotopes in the grain compared to the straw could be partly explained by vacuolar sequestration of Cd in the shoot, were light Cd isotopes may be complexed by thiols. In addition to these processes, the speciation of Cd and Zn in xylem and phloem sap could explain the different sense of isotope fractionation for these elements between straw and grains in grasses.[61] Speciation studies revealed that

Cd and Zn are mostly present as free ions[76, 77] in the xylem sap whereas most Cd is complexed[78] to ligands such as thiols[78, 79] and specific proteins[78] during transport in the phloem sap. While nicotianamine is thought to increase the intercellular mobility of Zn and the mobility of Zn in the alkaline phloem,[75] the role of thiols in the phloem sap for complexation Cd is unclear.[80] If the assumption that light Cd isotopes are preferentially complexed to PC's[50] is correct, the enrichment of heavy Cd isotopes in the grains suggests that Cd-PC complexes are not transported into the latter.

Cadmium isotope fractionation in plants

Analyses of birch leaves revealed an enrichment of heavy isotopes in such samples, with $\delta^{114/110}$Cd values ranging from 0.3 to 1.3 (Figure S6).[30] Whilst no Cd isotope measurements were carried out for the corresponding soils in this study, previous investigations reported only very limited Cd isotope variability for terrestrial silicate rocks with an inferred mean upper continental crust composition of $\delta^{114/110}$Cd = 0.0‰ (Figure S6).[81, 82] This suggests that Cd isotopes in birch leaves are also enriched in heavy isotopes compared to the bulk soils as in the present study. Contrasting results were obtained in recent studies, which investigated Cd isotope fractionation in cultivars of a Cd-tolerant tolerant plant (*Ricinus communis* L.) and in the Cd-hyperaccumulator *Solanum nigrum* L..[31, 32] The latter investigations found that the plants were enriched in light isotopes compared to the nutrient solution, whereby the Cd-hyperaccumulator revealed an enrichment of heavy isotopes in stem and leaves compared to the roots. However, the Cd-tolerant cultivars of *R. communis* displayed variable directions of Cd isotope fractionation between the different plant parts, which was interpreted to reflect different mechanisms of Cd accumulation within the plants.

433 A comparison of our study with these recent findings must consider the distinct nature of
434 three very different plant systems – a perennial culture, Cd-tolerant plants cultivated in
435 hydroponics at contaminated conditions, and a Cd-sensitive plant cultivated on non
436 contaminated soils. For example, the distinct fractionation of Cd isotopes between nutrient
437 solution and the whole plant for the Cd-tolerant and the Cd-sensitive plant could be a
438 consequence of the different cultivation techniques. However, the results obtained for all
439 three systems demonstrate that redistribution of Cd within different plant parts is associated
440 with distinct fractionation patterns. This might be the consequence of different transport or
441 retention mechanisms, which involve different ligands binding to Cd.[83] In summary, the
442 findings indicate that Cd isotope investigations of plants provide a novel tool, which can be
443 applied to study the different strategies of plants for coping with the toxic pollutant Cd,[69]
444 when this is introduced into soil-plant systems.

445

446 **Associated content**

447 Section 1 contains detailed information for material and methods; Table S1 summarizes Cd
448 isotope and concentration data for standard reference materials; Figure F1 presents DM
449 production, Cd concentrations and Cd uptake; Table S2 summarizes soil properties; Figure S2
450 and Table S3 summarize concentrations of macro- and micronutrients in different wheat
451 parts; Figure S3 relates Cd isotopes in the $Ca(NO_3)_2$ extract with Cd in the A horizon; Figures
452 S4-S5 present box models for Oen and LQ; Table S4 provides Cd isotope data ($\delta^{114/110}$Cd
453 values) for plant and soil; Figure S6 summarizes Cd isotope variation in terrestrial systems.

454

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Acknowledgements

This study was funded by the Swiss Parliament via the National Research Program (NRP) 69 "Healthy Nutrition and Sustainable Food Production" (SNSF Grant No. 406940_145195/1). We thank the farmers from the study sites for cooperation, Enrico Martinoia and Urs Feller for the discussion of the results, Barry Coles for assisting with the mass spectrometry at the MAGIC Labs, Walther Polit for the technical support of the plant microwave digestions and the technical staff of the Plant Nutrition Group at ETH Zurich for support in the greenhouse and in the laboratory.

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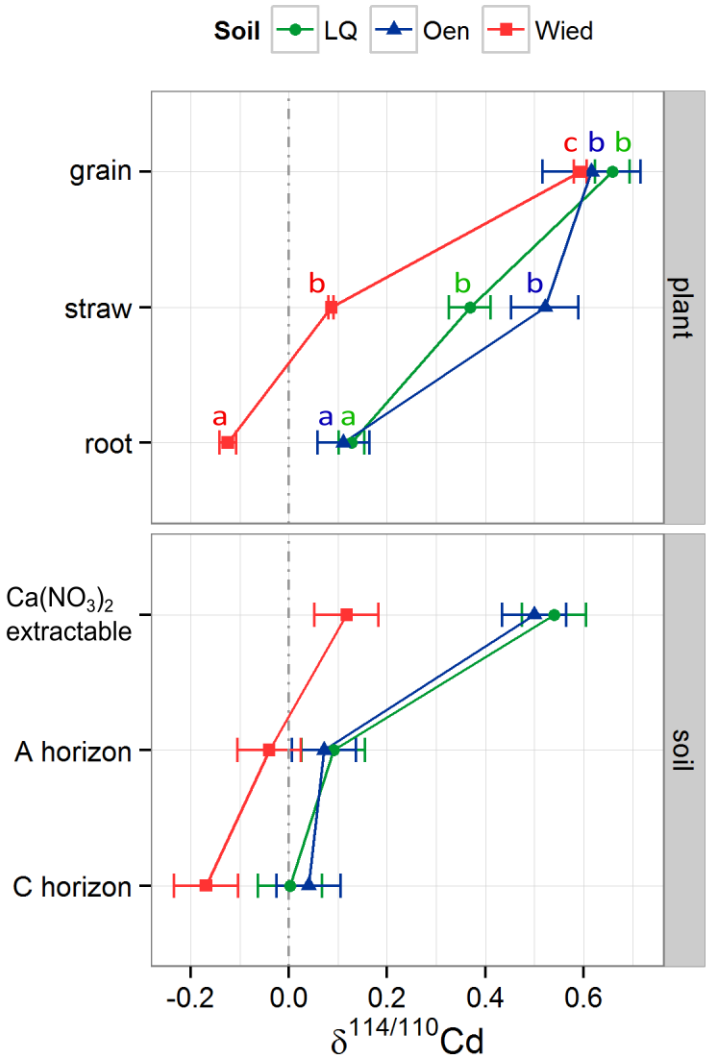


Figure 1: Cadmium stable isotope data, expressed as $\delta^{114/110}$Cd for soil-wheat systems. The connecting lines are used for illustrative purpose only. The site name abbreviations denote Oensingen (Oen), Wiedlisbach (Wied) and Landquart (LQ). Soil samples (lower box) were analyzed in analytical duplicate and the error bars represent ± 2sd. For plant samples (upper box), three individual replicates were analyzed, error bars represent ± 1se. The small letters indicate significant differences calculated with ANOVA followed by a Tukey HSD test.

Table 1: Cadmium uptake and $\delta^{114/110}$Cd values for whole wheat plants and shoots (straw + grain). Also shown is the isotopic fractionation $\Delta^{114/110}$Cd between the whole plants and shoots relative to the bulk A horizon and the $Ca(NO_3)_2$ extractable Cd.

	Soil	Cd uptake		$\delta^{114/110}$Cd		$\Delta^{114/110}$Cd		$\Delta^{114/110}$Cd	
		ng plant^{-1}	se	‰	se	‰ wheat-Ahorizon	se	‰ wheat-extract	se
whole plant (wheat)	Oen	1015	±336	0.46	±0.08	0.39	±0.08	0.03	±0.06
	LQ	908	±97	0.33	±0.01	0.16	±0.01	-0.21	±0.01
	Wied	1627	±77	0.09	±0.03	0.13	±0.02	-0.03	±0.02
		ng plant^{-1}	se	‰	se	‰ shoot-Ahorizon	se	‰ shoot-extract	se
shoot	Oen	776	±258	0.57	±0.09	0.50	±0.09	0.06	±0.07
	LQ	574	±41	0.45	±0.04	0.28	±0.01	-0.09	±0.04
	Wied	1053	±42	0.20	±0.01	0.24	±0.02	0.08	±0.005

SE = standard error of the mean of n = 3 individually grown plants, n=2 individually processed soil samples.

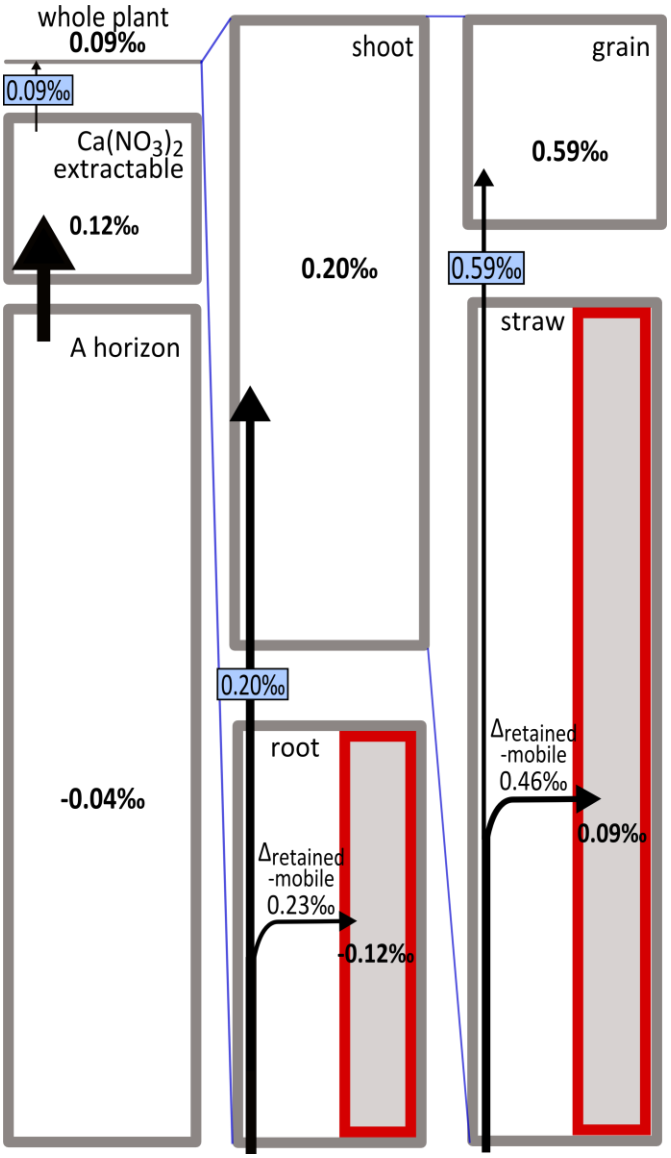


Figure 2: Box model that illustrates the Cd mass balance and isotope fractionation in the soil-wheat system at site Wied, (see Figures S4-5 for sites Oen and LQ). Numbers in ‰ represent average $\delta^{114/110}$Cd values. The δ-values in the blue boxes represent the exported δ-values from a soil pool or plant part (e.g., root) to the next soil pool or plant part (e.g., shoot). The exported δ-values are identical to the δ-value measured for the next soil pool or plant part (e.g., shoot). The $\Delta^{114/110}$Cd values were modeled with data from Wied, but also Oen and LQ as shown in Figure 3 and represent the apparent Cd isotope fractionation that results from retention of Cd in the respective plant part. The width of the arrows and size of the boxes are adapted to denote the relative sizes of the Cd fluxes and Cd pools, respectively.

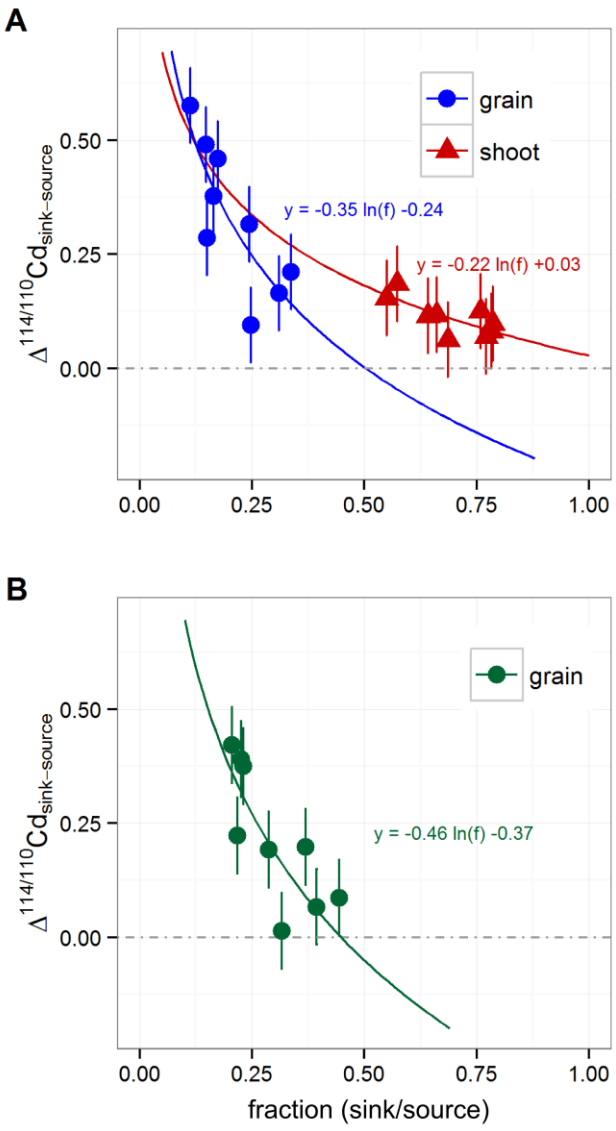
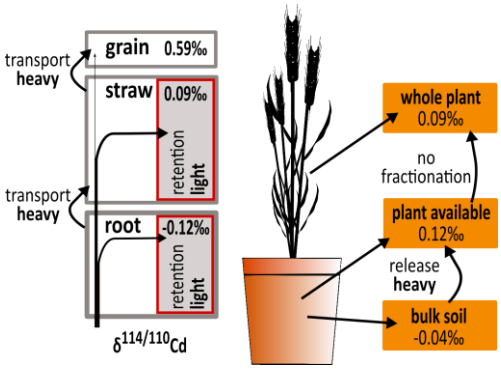


Figure 3: Cadmium isotope fractionation in soil-wheat systems. In (a), values on the x-axis denote the mass fraction of Cd stored in shoots or grains (sink) relative to the mass of Cd taken up by the whole plant (source). In (B), the Cd in the shoots serves as the source and the grains as sink. The y-axes represent the isotopic fractionation $\Delta^{114/110}$Cd between the same sinks and sources that are applied for the x-axes. Each point represents an individual plant and error bars denote the 2sd reproducibility of ±0.083‰ obtained for plants. The lines represent the results of Rayleigh fractionation models discussed in the text.

Graphical Abstract



Cadmium isotope fractionation in soil-wheat systems

Supporting Information

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Contents

1. Materials and Methods (detailed information) 4

1.1. Plant growth experiment 4

1.2. Sample preparation 4

1.3. Analyses and standard reference materials 5

2. Cadmium isotope compositions and concentrations of standard reference materials (SRMs) 7

3. Soil properties of Oensingen (Oen), Landquart (LQ) and Wiedlisbach (Wied). 8

4. DM production, Cd concentration and Cd uptake of wheat 9

5. Distribution of Cd, macro and micronutrients in wheat 10

6. Apparent isotope fractionation between A horizon and $Ca(NO_3)_2$ extract 14

7. Cd isotope compositions (as $\delta^{114/110}$Cd values) of the soil–plant systems 15

8. Box model Oensingen 16

9. Box model Landquart 17

10. Comparison with published Cd isotope data for terrestrial and aquatic environments 18

12. Literature 20

Tables

Table S1: Summary standard reference materials 7

Table S2: Soil properties 8

Table S3: Element concentrations measured in the different wheat parts. 11

Table S4:: Cadmium isotope compositions (as $\delta^{114/110}Cd$) of the soil-wheat systems. 15

Figures

Figure S1: Dry matter production, Cd concentration and Cd uptake of wheat 9

Figure S2: Relative distribution of Cd, macro and micronutrients in wheat 10

Figure S3: $\Delta^{114/110}Cd_{extract\text{-}Ahorizon}$ versus the mass fraction of $Ca(NO_3)_2$ 14

Figure S4: Boxplot Oensingen 16

Figure S5: Boxplot Landquart 17

Figure S6: Literature comparison 19

1. Materials and Methods (detailed information)

1.1. Plant growth experiment

To ensure regular plant growth, 100 mg N (80 mg N applied as $Ca(NO_3)_2$ and 20 mg as NH_4NO_3), 35 mg P (KH_2PO_4), 50 mg K (K_2SO_4), 25 mg Mg ($MgSO_4$), 5 mg Fe (Fe-EDTA) and 2 mg Zn ($ZnSO_4$) were added to each pot and mixed thoroughly.

The wheat plants were cultivated in a growth chamber with the following settings: photoperiod 16 h, illuminance 25 klx, temperature 24°C at daytime and 16°C at night, humidity 60%. The wheat was watered to maintain a range of 40 to 70% of the maximal water holding capacity (Table S2).

1.2. Sample preparation

The roots were rinsed with 6 mM $NaNO_3$ and transferred for 15 min into a 6 mM $NaNO_3$ cleaning bath followed by rinsing with the same solution. Cleaning bath (5 min) and rinsing were repeated up to three times until the roots and waste cleaning solution were visibly free of any soil particles. Sodium nitrate was chosen to disperse the clay minerals that adhered to the roots.[1] The concentration of the cleaning solution was adapted to the equivalent of the conductivity[2] in soil solutions obtained in currently running field studies at the same study sites (own, unpublished results).

To digest the plants in the high pressure single reaction chamber microwave system (turboWave, MWS microwave systems), 1 g portions of material were treated using 12 ml 8 M HNO_3. The A and C horizon of the soils were digested using a mixture of 8 ml 16 M HNO_3 and 2 ml 24 M HF (Milestone, SK-10 high pressure rotor). Several digestion batches of one sample were combined to achieve a minimum quantity of 100 ng Cd for isotopic analysis.

1.3. Analyses and standard reference materials

Application of the double spike methodology for the Cd isotope analyses ensured that results were unbiased by any isotope fractionation induced by the Cd separation chemistry and enabled precise correction for the large instrumental mass bias incurred in MC-ICP-MS. Additionally, use of the double spike facilitated precise determination of Cd concentrations by isotope dilution (ID).[3] To this end, an appropriate volume of the ^{111}Cd - ^{113}Cd double spike solution was equilibrated with the samples after digestion.[4] Cadmium was then separated from the sample matrix using a three-stage separation chemistry that employs both anion exchange and extraction chromatography.[4, 5] This was followed by a liquid-liquid extraction step for sample cleanup.[5]

Cadmium concentrations determined by ID-MC-ICP-MS for plant and soil samples (n = 2–3) were employed to investigate the relationship between Cd pool sizes and Cd isotope fractionation (Table 1, Figure 3, Figure S3). The Cd concentrations of all plant samples were also measured by quadrupole ICP-MS without prior matrix separation. These results exceeded the Cd abundances determined by ID-MC-ICP-MS by 7.5 ± 2.0% (1se). The quadrupole ICP-MS Cd data were used to compare the Cd concentrations and Cd quantities of plant samples (n = 4; Figure S1).

A number of standard reference materials (SRMs) were analyzed during the course of the study for quality control, with results summarized in Table S1. The performance of the MC-ICP-MS instrument was routinely monitored based on multiple analyses of SRM BAM I012 Cd. These measurements yielded $\delta^{114/110}$Cd = -1.30 ± 0.09 (2sd, n = 12), in good agreement with the consensus value of -1.33 ± 0.04.[6]

The Cd isotope compositions and contents of three geological and biological SRMs with matrices and Cd concentrations similar to those of the experimental samples were also analyzed. These were andesite rock USGS SRM AGV-2, soil NIST SRM 2709a, and wheat flour NIST SRM 1567b (Table S1). The Cd concentrations of the SRMs were determined using the added double spike for ID calculations.[3] The Cd isotope data acquired for these SRMs showed good reproducibilities of

between 0.02‰ and 0.11‰ (2sd, n=2–6), as determined from multiple analyses of separately treated sample aliquots (Table S1). Isotopic reference data are unfortunately not available for these SRMs.

The Cd concentrations obtained for the SRMs are indicative of good Cd recoveries for soil NIST SRM 2709a (96 ± 3%, 2sd) and rock USGS SRM AGV-2. For the latter sample, recent independent analyses conducted at Imperial College yielded a Cd concentration of 67.7 ng g^{-1} (own, unpublished result), in excellent agreement with the present result of 66.6 ± 0.03 ng g^{-1} (2sd, n=2). Notably, other recent studies reported Cd contents of between about 60 and 3000 ng g^{-1} for this sample, indicating that Cd may be heterogeneously distributed in the powder.[7] There is, however, a small but clearly resolvable discrepancy between the Cd concentration determined here for wheat flour NIST 1567b and the reference value. In detail, our analyses indicate a Cd recovery of only 88 ± 8% (2sd, n = 6). The Cd recovery for this SRM did not improve when initially undigested silicates were treated with HF for full dissolution. The low recovery may hence reflect a slightly erroneous reference value for this sample. This conclusion is based on the observation that the certified Cd concentration of 254 ± 0.9 ng g^{-1} was not determined on the actual NIST SRM 1567b material but adopted from the Cd concentration measured for the original NIST 1567a sample.[8]

2. Cadmium isotope compositions and concentrations of standard reference materials (SRMs)

Table S1: Summary standard reference materials

SRM	Type	$\delta^{114/110}$Cd ± 2sd[a] (‰)	Reference (‰)	Cd conc. ± 2sd[a] (µg g^{-1})	Reference (µg g^{-1})	n
NIST 2709a	Soil	-0.22 ± 0.02	-	0.356 ± 0.01	0.371	2
NIST 1567b	Wheat flour	0.93 ± 0.08	-	0.0216 ± 0.0019	0.0254	6
USGS AGV-2	Andesite rock	0.11 ± 0.11	-	0.0666 ± 0.0003	0.063-0.34[c]	2[b]
BAM-I012	Isotopic RM	1.30 ± 0.09	-1.33 ± 0.04[d]	-	-	12

[a] 2sd = 2 × standard deviation of n samples.
[b] A single sample was digested and spiked and then split into two samples for which column chemistry and isotopic analyses were done separately.
[c] Values summarized from GeoReM[7]
[d] Mean value from Abouchami et al. (2013)[6]

3. Soil properties of Oensingen (Oen), Landquart (LQ) and Wiedlisbach (Wied).

Table S2: Soil properties

General information				A horizon [a]								C horizon [b]	
Origin	soil type[9]	Land use	texture	max. water holding capacity	CEC	pH	C-content	total Cd		$Ca(NO_3)_2$ extractable Cd [c]		total Cd	
				g H_2O/g soil	$mmol_c kg^{-1}$	($CaCl_2$)	g kg^{-1}	ng g^{-1}	se [d]	ng g^{-1}	se [d]	ng g^{-1}	se [d]
Oen	Cambisol	arable farming	silt loam	0.51	164	5.6	24	508	±20	20.4	±9.8	152	± 0.4
LQ	Fluvisol	arable farming	silt loam	0.60	217	7.1	57	287	±3	2.75	±0.25	129	± 4
Wied	Cambisol	arable farming	sandy loam	0.38	44	5.2	15	193	±40	39.6	±3.9	113	± 11

[a] The A horizons were sampled from 0-20 cm.
[b] The C horizons were provided by the Swiss National Soil Observation Program (NABO) from their archive.
[c] Plant-available Cd was extracted using 0.05 M $Ca(NO_3)_2$.
[d] se denotes standard error of the mean (n=2).

4. DM production, Cd concentration and Cd uptake of wheat

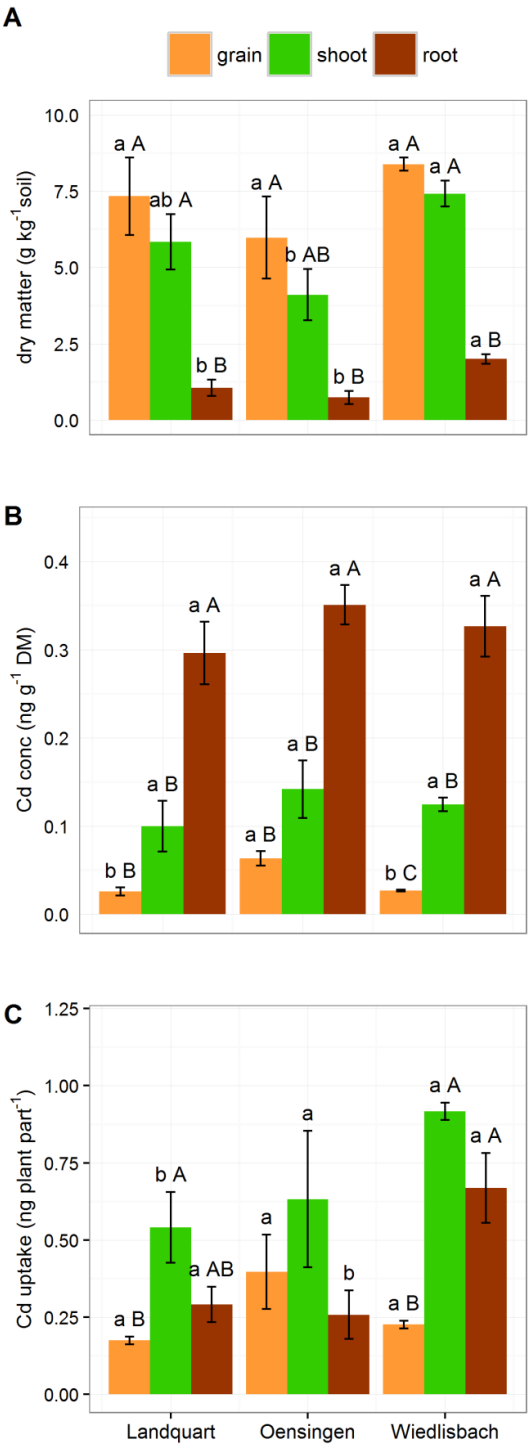


Figure S1 (A) Dry matter production, (B) Cd concentration and (C) Cd uptake of wheat grown on different soils under controlled conditions. Error bars indicate standard error (n=4 for LQ and Wied, n=3 for Oen). Upper case letters denote significant differences of the mean between different plant parts from wheat grown on the same soils. Lower case letters indicate significant differences between the same plant parts grown on different soils ($p < 0.05$).

5. Distribution of Cd, macro and micronutrients in wheat

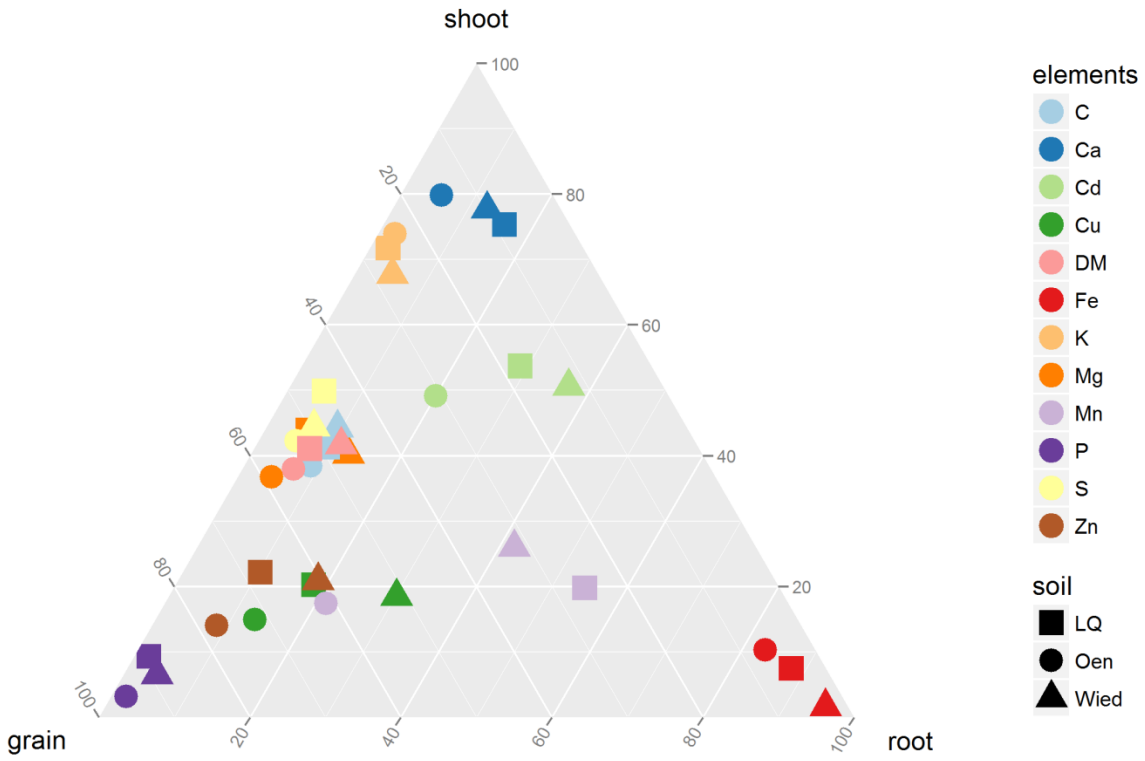


Figure S2: Relative distribution of Cd, macro and micronutrients in the analyzed plant parts of wheat. Colors represent the elements measured and shapes represent the different soils. The values are given in percent, as calculated from the mean values (quantity of element as ng per plant part, n=4). Cd, Cu and Zn were measured by ICP-MS; Ca, Fe, K, Mg, Mn and P were measured by ICP-OES; C and S by IRMS.

Table S3: Element concentrations measured in the different wheat parts.

element	unit	mean[b]	standard error[a]	soil	plant part
C	g/kg DM	416	1	LQ	grain
		414	2	Oen	
		417	0	Wied	
		439	3	LQ	straw
		441	1	Oen	
		451	1	Wied	
		415	1	LQ	root
		435	3	Oen	
		360	19	Wied	
Ca	g/kg DM	0.49	0.03	LQ	grain
		0.56	0.03	Oen	
		0.41	0.04	Wied	
		5.40	0.40	LQ	straw
		4.30	0.50	Oen	
		3.75	0.22	Wied	
		5.87	0.57	LQ	root
		1.72	0.13	Oen	
		2.25	0.17	Wied	
Cd	mg/kg DM	0.026	0.005	LQ	grain
		0.063	0.008	Oen	
		0.027	0.001	Wied	
		0.100	0.029	LQ	straw
		0.142	0.033	Oen	
		0.125	0.008	Wied	
		0.296	0.035	LQ	root
		0.351	0.022	Oen	
		0.327	0.035	Wied	
Cu	mg/kg DM	6.33	0.76	LQ	grain
		5.11	0.93	Oen	
		4.28	0.05	Wied	
		2.58	0.23	LQ	straw
		1.45	0.10	Oen	
		1.73	0.06	Wied	
		13.44	1.37	LQ	root
		6.97	0.43	Oen	
		10.49	0.61	Wied	
Fe	mg/kg DM	18	2	LQ	grain
		26	2	Oen	

element	unit	mean	standard error[a]	soil	plant part
Fe	mg/kg DM	35	1	Wied	grain
		42	16	LQ	straw
		46	20	Oen	
		23	6	Wied	
		2280	254	LQ	root
		2567	370	Oen	
		4708	392	Wied	
K	g/kg DM	3.9	0.4	LQ	grain
		4.8	0.4	Oen	
		4.0	0.2	Wied	
		13.9	1.3	LQ	straw
		21.2	1.8	Oen	
		11.4	1.2	Wied	
		2.5	0.4	LQ	root
		3.9	0.3	Oen	
		3.0	0.4	Wied	
Mg	g/kg DM	1.2	0.1	LQ	grain
		1.2	0.0	Oen	
		1.3	0.0	Wied	
		1.3	0.1	LQ	straw
		1.0	0.2	Oen	
		1.3	0.1	Wied	
		0.9	0.1	LQ	root
		0.8	0.1	Oen	
		1.5	0.1	Wied	
Mn	mg/kg DM	2.6	0.4	LQ	grain
		13	0.7	Oen	
		34	1.6	Wied	
		3.2	0.3	LQ	straw
		5.0	0.8	Oen	
		31	6	Wied	
		39	5	LQ	root
		40	7	Oen	
		185	10	Wied	
N	g/kg DM	20.2	1.5	LQ	grain
		20.0	0.9	Oen	
		18.2	0.3	Wied	
		7.6	0.4	LQ	straw
		6.3	1.0	Oen	
		6.9	0.0	Wied	

element	unit	mean	standard error[1]	soil	plant part
P	g/kg DM	3.53	0.52	LQ	grain
		2.74	0.47	Oen	
		3.16	0.09	Wied	
		0.48	0.12	LQ	straw
		0.12	0.01	Oen	
		0.26	0.02	Wied	
		0.55	0.07	LQ	root
		0.41	0.06	Oen	
		0.65	0.06	Wied	
S	g/kg DM	1.66	0.10	LQ	grain
		1.52	0.07	Oen	
		1.55	0.02	Wied	
		2.45	0.56	LQ	straw
		1.76	0.12	Oen	
		1.58	0.06	Wied	
		0.88	0.06	LQ	root
		0.92	0.02	Oen	
		0.83	0.03	Wied	
Zn	mg/kg DM	23.9	4.0	LQ	grain
		18.9	0.8	Oen	
		25.3	0.9	Wied	
		9.9	1.5	LQ	straw
		4.9	0.4	Oen	
		9.9	0.8	Wied	
		26.2	4.3	LQ	root
		17.6	1.6	Oen	
		32.0	2.1	Wied	
DM	g	7.3	1.3	LQ	grain
		6.0	1.3	Oen	
		8.4	0.2	Wied	
		5.8	0.9	LQ	straw
		4.1	0.8	Oen	
		7.4	0.4	Wied	
		1.1	0.3	LQ	root
		0.7	0.2	Oen	
		2.0	0.2	Wied	

[a] *Standard error of the mean of n= 4 samples*

[b] *Cd, Cu and Zn were determined by ICP-MS; Ca, Fe, K, Mg, Mn and P were determined by ICP-OES; C, N and S by IRMS.*

6. Apparent isotope fractionation between A horizon and $Ca(NO_3)_2$ extract

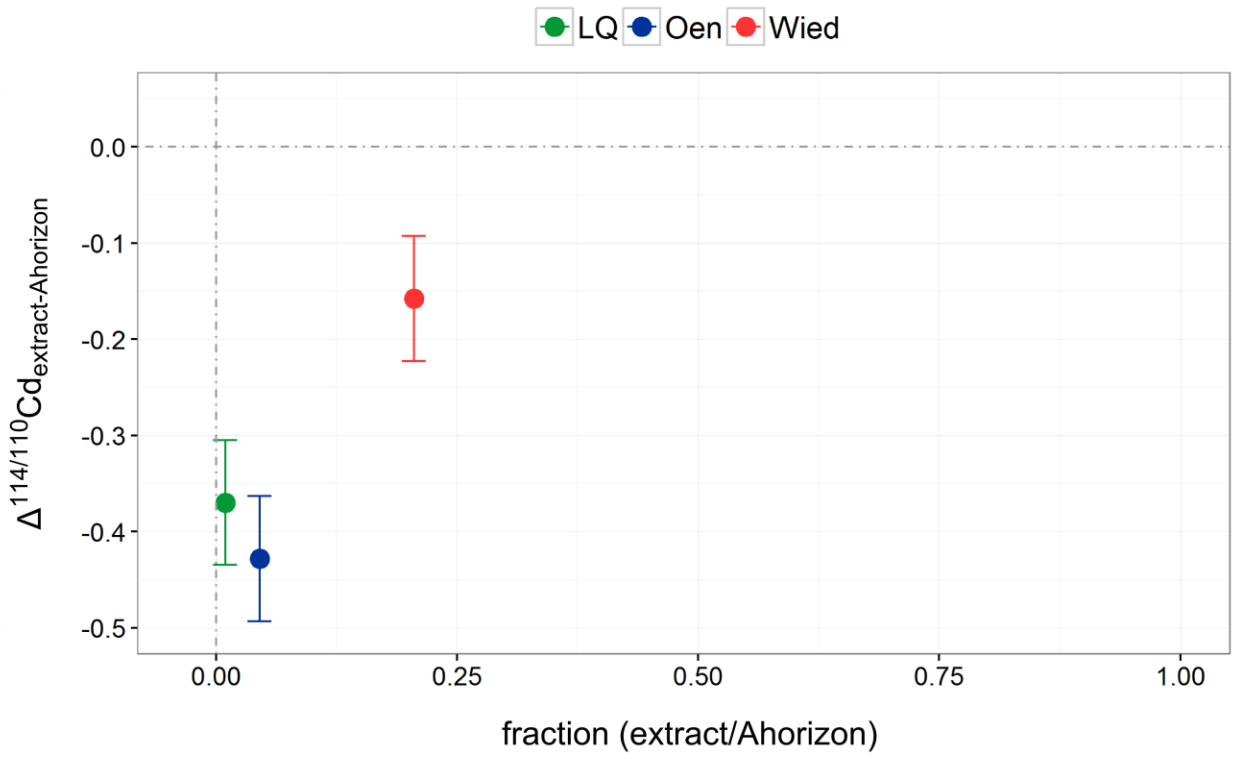


Figure S3: Relationship between the fractionation $\Delta^{114/110}Cd_{extract\text{-}Ahorizon}$ and the mass fraction of $Ca(NO_3)_2$ extractable Cd in soil (relative to the total mass of Cd in the A horizon)

7. Cd isotope compositions (as $\delta^{114/110}$Cd values) of the soil–plant systems

Table S4: Cadmium isotope compositions (as $\delta^{114/110}$Cd) of the soil-wheat systems.

	Oen		LQ		Wied	
	$\delta^{114/110}$Cd ‰	SE	$\delta^{114/110}$Cd ‰	SE	$\delta^{114/110}$Cd ‰	SE
grain	0.62	±0.10	0.66	±0.04	0.59	±0.01
straw	0.52	±0.07	0.37	±0.04	0.09	±0.01
root	0.11	±0.05	0.13	±0.03	-0.12	±0.02
$Ca(NO_3)_2$ extractable	0.50	±0.05	0.54	±0.05	0.12	±0.05
A horizon	0.07	±0.01	0.09	±0.01	-0.04	±0.01
C horizon	0.04	±0.02	0.00	±0.02	-0.16	±0.02

SE = standard error of the mean, where n = 3 for plant samples and n = 2 for soil samples

8. Box model Oensingen

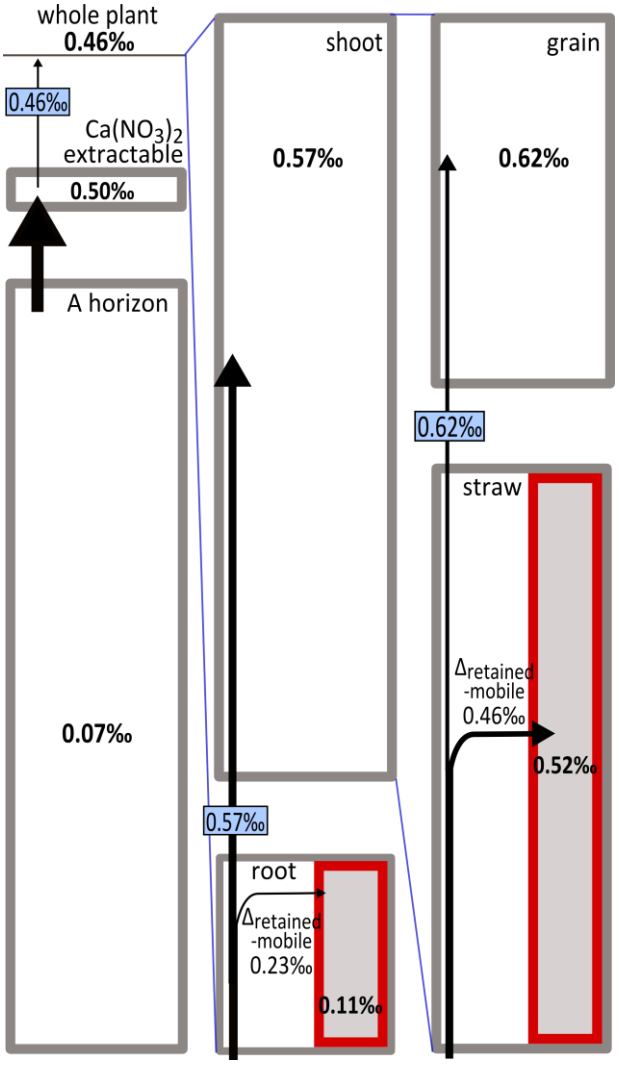


Figure S4: Box model that illustrates the Cd mass balance and isotope fractionation for the soil-wheat system at site Oensingen (Oen). Numbers in ‰ represent average $\delta^{114/110}$Cd values. The δ-values in the blue boxes represent the exported δ-values from a soil pool or plant part (e.g., root) to the next soil pool or plant part (e.g., shoot). The exported δ-values are identical to the δ-value measured for the next soil pool or plant part (e.g., shoot). The $\Delta^{114/110}$Cd values were modeled with data from Oen, but also Wied and LQ as shown in Figure 3 and represent the apparent Cd isotope fractionation that results from retention of Cd in the respective plant part. The width of the arrows and size of the boxes are adapted to denote the relative sizes of the Cd fluxes and Cd pools, respectively.

9. Box model Landquart

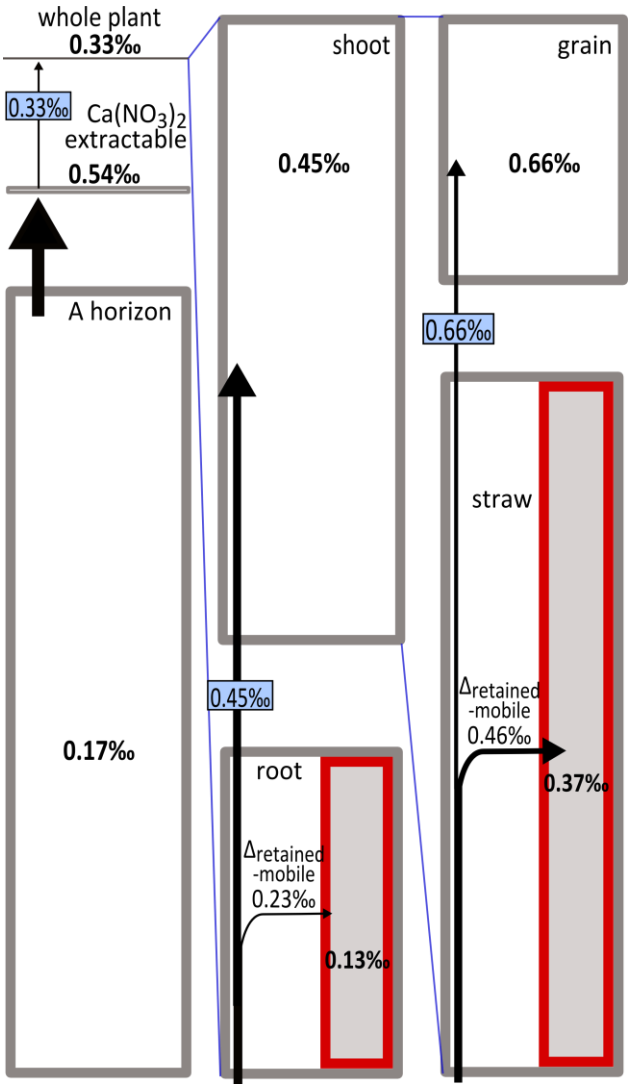


Figure S5: Box model that illustrates the Cd mass balance and isotope fractionation for the soil-wheat system at site Landquart (LQ). Numbers in ‰ represent average $\delta^{114/110}$Cd values. The δ-values in the blue boxes represent the exported δ-values from a soil pool or plant part (e.g., root) to the next soil pool or plant part (e.g., shoot). The exported δ-values are identical to the δ-value measured for the next soil pool or plant part (e.g., shoot). The $\Delta^{114/110}$Cd values were modeled with data from LQ, but also Wied and Oen as shown in Figure 3 and represent the apparent Cd isotope fractionation that results from retention of Cd in the respective plant part. The width of the arrows and size of the boxes are adapted to denote the relative sizes of the Cd fluxes and Cd pools, respectively.

10. Comparison with published Cd isotope data for terrestrial and aquatic environments

Figure S6 compares previously reported Cd isotope compositions for various terrestrial and aquatic environments with the plant data acquired in the present study. The comparison illustrates that rocks of the earth's mantle and crust, including fresh water sediments, show only limited isotopic variability with $\delta^{114/110}$Cd values of about -0.4‰ and 0.4‰. In contrast, seawater and marine sediments and deposits show a much wider range in $\delta^{114/110}$Cd, with values from -0.45‰ to about 5‰. The most positive $\delta^{114/110}$Cd values are found in surface seawater, where dissolved Cd concentrations are extremely low, due to the uptake and storage of isotopically light Cd by phytoplankton.[3, 10-12]

In our study, samples in which Cd isotope fractionation was dominated by biological processes also showed a larger isotopic variation than samples in which Cd isotope fractionation was dominated by abiotic geological processes. The A and C soil horizons displayed a much smaller range of $\delta^{114/110}$Cd (from -0.09 to -0.16‰) than the different wheat parts, which were characterized by $\delta^{114/110}$Cd = -0.12 to 0.97‰. An important additional source of Cd isotope variation in the investigated soil-plant systems is the apparent fractionation between the A horizon and the $Ca(NO_3)_2$ extractable Cd of the soil (Figure 1). Notably, published Cd isotope data for birch leaves[13] and Cd-tolerant plants[14] exhibit a similar range of $\delta^{114/110}$Cd values (from -0.39 to 1.3‰) as were measured here for the plant parts (-0.12 to 0.97‰).

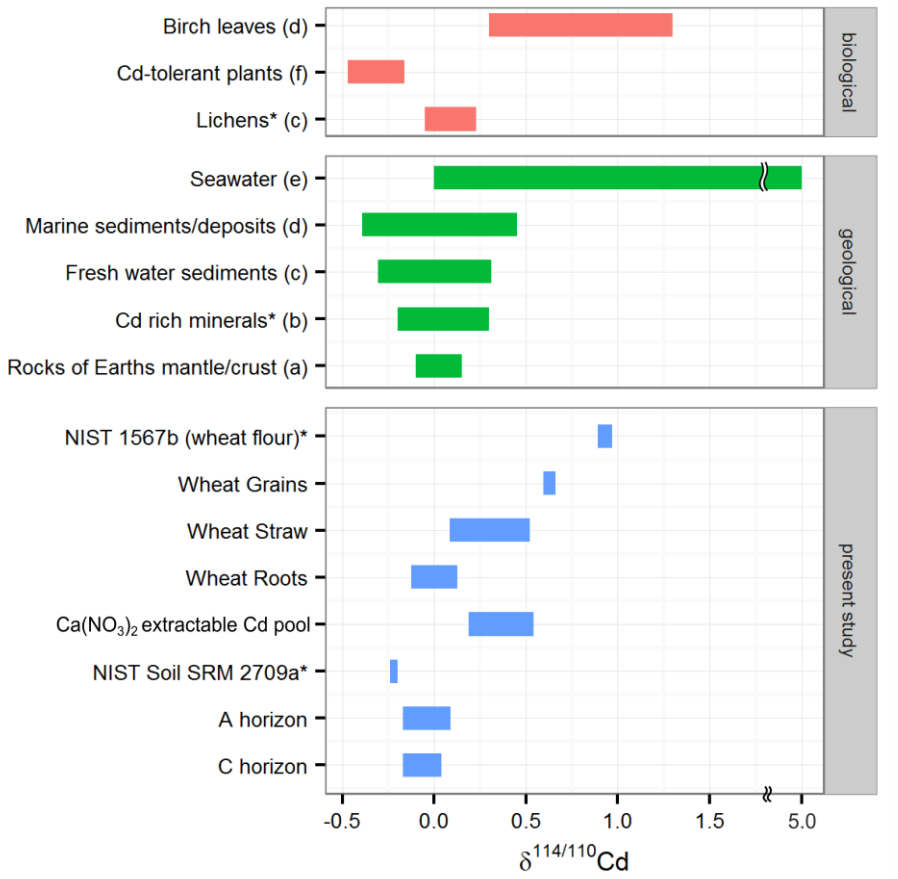


Figure S6: Summary of stable Cd isotope data ($\delta^{114/110}$Cd) for uncontaminated material in terrestrial and aquatic systems. Values are separated into biological and geological data and results obtained in the present study. Shown are the $\delta^{114/110}$Cd ranges of the samples unless the material (Y-axis) is marked with an asterisks, which indicates that the standard deviation of the dataset is plotted. All results are given relative to $\delta^{114/110}Cd_{NIST3108}$ = 0.[12] Small letters refer to the data sources.

Sources: (a) Schmitt et al.[15], Rehkämper et al.[16], (b) Schmitt et al.[15], Rehkämper et al.[16], Wombacher et al.[17], Shiel et al.[18], (c) Pallavicini et al.[13], (d) Schmitt et al.[15], Horner et al.[19], (e) Ripperger et al.[3], Lacan et al.[10], Conway & John[12], Abouchami et al.[20], Xue et al.[11], Rehkämper et al.[16], (f) Wei et al.[14], Wei et al.[21]

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