Using isotopes to trace freshly applied cadmium through mineral phosphorus fertilization in soil-fertilizer-plant systems

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Abstract

Applications of mineral phosphorus (P) fertilizer can lead to cadmium (Cd) accumulation in soils and can increase Cd concentrations in edible crop parts. To determine the fate of freshly applied Cd, a Cd source tracing experiment was conducted in three soil-fertilizer-wheat systems by using a mineral P fertilizer labeled with the radio isotope $^{109}$Cd and by exploiting natural differences in Cd stable isotope compositions ($\delta^{114/110}$Cd). Source tracing with stable isotopes overestimated the proportion of Cd in plants derived from the P fertilizer, because the isotope ratios of the sources were not sufficiently distinct from those of the soils. Despite indistinguishable extractable Cd pools between control and treatments, the addition of P fertilizer resulted in a more negative apparent isotope fractionation between soil and wheat. Overall, the radio isotope approach provided more robust results and revealed that 6.5 to 15 % of the Cd in the shoot derived from the fertilizer. From the introduced Cd, a maximum of 2.2 % reached the wheat shoots, whilst 97.8 % remained in the roots and soils. The low recoveries of the fertilizer derived Cd suggest that continuous P fertilizer application in the past decades can lead to a build-up of a residual Cd pool in soils.

Key Words: Cadmium, mineral P fertilizer, radio isotopes, source tracing, stable isotopes, wheat, pot experiment
1. Introduction

The toxic element Cd occurs naturally in soils, but it has been additionally released into the environment through human activities. A major concern is posed by the unintended input of Cd into arable soils through mineral P fertilization (Jiao et al., 2012). Cadmium can substitute for Ca$^{2+}$ in apatite (Ca$_{10-n}$ (PO$_4$)$_6$(OH,F), Fedoroff et al., 1999; Pan and Fleet, 2002; Sery et al., 1996). The latter is used to produce P fertilizer that contains, compared to the initial product, readily soluble Ca(H$_2$PO$_4$)$_2$ and/or CaSO$_4$ and unintentionally also soluble Cd (Fedoroff et al., 1999; Prochnow et al., 2008). In Switzerland, the average Cd concentrations of P fertilizers are about 22 times higher than in arable soils (Bigalke et al., 2016; FOAG, 2015). Consequently, application of P fertilizer can lead to Cd accumulation in arable top soils (Bigalke et al., 2016) and to an increase of Cd in staple food products (Grant et al., 2013). In human nutrition, Cd poses a threat since it has a long biological half-life of about 10 years and damages organs such as the kidney (Godt et al., 2006). In this context, the fate of fertilizer-derived Cd and its impact on Cd transfer from soil to edible plant parts needs to be well understood in order to characterize the risk of Cd transfer into the food chain.

Isotope source tracing techniques are commonly used to investigate the fate of nutrients that were applied with fertilizer (e.g., Bosshard et al., 2009; McBeath et al., 2013; Nanzer et al., 2014). Compared to nutrient mass balances, isotope tracing of freshly applied nutrients provides further insights into the fate of nutrients by not only taking into account inputs- and outputs of soil-fertilizer-
plant systems but also the fluxes and dynamics within these systems. However, to our knowledge only one study used isotopes (the radio isotope $^{109}$Cd) to trace fertilizer-derived Cd in soil-plant systems (Jensen and Mosbæk, 1990). It revealed that 4 to 16% of the Cd added to the soil-lettuce system with NPK-fertilizer reached the plants, such that the majority of the applied Cd remained in the soil. To date, no such study exists for wheat although it often receives Cd through mineral P fertilizers and wheat is one of the main Cd contributors in the diet of western European countries (Filippini et al., 2018; Vromman et al., 2010).

Recent advances in metal stable isotope analysis enabled the use of isotope ratios at natural abundance to trace sources of metals in the environment (Wiederhold, 2015). The application of isotope ratios at natural abundances is particularly interesting since the element sources of the investigated system do not need to be additionally processed for Cd labeling, so that one manipulation step that potentially creates artifacts can be skipped. Furthermore, the approach does not require the purchase of isotope spikes, which significantly increase costs depending on the size of the experiment and the scale of observation. However, the contribution of different sources (e.g., soil, fertilizer) to a sink (e.g., grain) can only be determined with confidence if (i) the sources have distinct isotope ratios, (ii) the extent of isotope fractionation that occurs in the system (e.g., during plant uptake and transport processes) is known or negligible relative to the isotopic difference between the sources and (iii) additional sources can be excluded. Two recent studies reported on the use of
stable isotopes at natural abundance to determine the impact of past anthropogenic Cd inputs on the current Cd stocks in soils. Salmanzadeh et al. (2017) showed that the Cd stock of arable soils can be dominated by past applications of mineral P fertilizers. This finding is based on distinct isotope ratios of old and new mineral P fertilizers, which governed the Cd isotope compositions in the soils of a long-term field trial in New Zealand. Imseng et al. (2018) measured Cd isotope ratios for all relevant inputs- (atmospheric deposition, mineral and organic fertilizer, weathering), outputs (crop offtake, leaching) and sinks (soil) of three arable cropping systems. Cadmium mass balances and isotope ratios revealed that long-term processes such as soil weathering and soil-plant cycling determined the current distribution of Cd isotopes in soils whilst recent anthropogenic Cd inputs played a minor role.

Cadmium isotope ratios at natural abundance may also provide novel insights into the fate of freshly applied Cd in soil-fertilizer-plant systems. Recent studies have shown that the Cd isotope ratios of mineral P fertilizers ($\delta^{114/110}$Cd) range between -0.15 ‰ and 0.15 ‰ (Imseng et al., 2018) while the plant-available Cd pool (obtained using Ca(NO$_3$)$_2$ extraction) of arable Swiss soils ranged from 0.12 ‰ to 0.54 ‰ (Wiggenhauser et al., 2016). Furthermore, no or only minor Cd isotope fractionation was observed between the plant-available Cd pool of the arable soils and whole wheat plants ($\Delta^{114/110}$Cd$_{whole\ plant-extractable}$ -0.21 to 0.03 ‰). Thus, a source tracing approach using stable isotopes at natural abundance would be applicable to selected soil-fertilizer-wheat systems, if the
addition of soluble mineral P fertilizer shifts the plant-available Cd pool towards lighter isotopes.

In this study, Cd was freshly applied with a mineral P fertilizer and traced in soil-wheat systems. This was achieved both with the aid of a Cd radio isotope and stable isotope ratios at natural abundance, which were both employed in Cd mass balances calculations. We aimed to (i) test if the natural variation of Cd stable isotopes in the Cd sources of the plant (soil, fertilizer) can be used to trace the distribution of freshly applied Cd in a closed soil-fertilizer-wheat system and (ii) to quantify the fate of freshly applied Cd derived from mineral P fertilization in soil-wheat systems.

2. Material & Methods

2.1. Soil-fertilizer-wheat systems

Three soils from Oensingen (Oen), Wiedlisbach (Wied) and Landquart (LQ) were selected to represent typical arable soils for wheat production in Switzerland. The soils have distinct properties (Table A1) such as pH, texture and C concentration which influence the size of the plant-available Cd pool (Smolders and Mertens, 2013). For two of the three soils (Oen, Wied), Cd mass balances at the field scale were determined recently (Imseng et al., 2018). The third soil from LQ was chosen because of its comparatively high pH (7.1). In Oen, we measured stable isotopes in soils and wheat that had received unlabeled mineral P fertilizer. The wheat cultivar (Triticum aestivum L., cv. "Fiorina") has been commonly cultivated in the past years in Switzerland. A
commercially available mineral P fertilizer (triple super phosphate, TSP) was chosen with a Cd concentration of 19 mg kg\(^{-1}\) Cd per kg of dry weight (equivalent to 92 mg kg\(^{-1}\) P) which is at the upper end of Cd concentrations for mineral P fertilizers applied to Swiss agricultural soils (FOAG, 2015). We applied 500 mg mineral P fertilizer with 9.5 μg Cd (Table A2) to 1 kg of soil. This represents a P fertilization of 230 kg ha\(^{-1}\) y\(^{-1}\), an amount of P that is about 10 times larger than the current average P-fertilization of arable soils in Switzerland. However, P fertilization in Switzerland peaked in the 1970s and was then reduced by 80 % until 2005 (Spiess, 2011). Thus, the chosen model soil-fertilizer-wheat system can be considered as a worst-case scenario for Swiss farming practice in terms of unintended Cd addition to soils.

2.2. **Fertilizer labeling**

We adapted the protocol of Jensen and Mosbæk (1990) to label mineral P fertilizer with a radioactive Cd isotope. The P fertilizer (6.5g) was dissolved in deionized water (130mL, >18.2 MΩ) and continuously stirred. Afterwards, carrier-free \(^{109}\)Cd (CdCl\(_2\) in 0.5M HCl, 6MBq, radio isotope center POLATOM) was added to the mineral P fertilizer suspension, stirred for 10 min and evaporated slowly to dryness on a hotplate. The labeled fertilizer was then carefully ground and split into 13 portions, each containing 0.5 g of labeled fertilizer. Twelve portions were used for the pot experiment and one portion was used to measure the specific activity of the mineral P fertilizer added to each pot. For characterization, we compared the mineralogy of the inorganic crystallized species in the original mineral P fertilizer with the evaporated
fertilizer using Powder X-ray Diffraction (XRD, Bruker AXS D8 Advance). The comparison revealed that the described procedure did not alter the Ca speciation of the fertilizer (Fig. A1). Furthermore, the Cd water solubility of the fertilizer was determined before and after evaporation. To this end, a solid to liquid ratio of 1:10 with deionized H₂O (>18.2 MΩ) as extractant was used, the reaction time was 2h on a horizontal shaker, where after the suspension was passed through a paper filter (ashless filter, No. 42, Machery-Nagel). The results showed that the labeling process did not change the water solubility of Cd in the mineral P fertilizer (Table A2).

To test if the fertilizer addition to the soil changed the Cd isotope composition of the plant-available Cd pool, a 0.05M Ca(NO₃)₂ extract was applied to soils that had received mineral P fertilizer following the protocol reported previously (Wiggenhauser et al., 2016).

2.3. Pot experiment and sample processing
Wheat was cultivated in the same way and period as published previously (Wiggenhauser et al., 2016). Briefly, the pots where filled with 1 kg of dry soil, macro and micro nutrients were added to ensure regular plant growth before the wheat was sown. For the treatments that received mineral P fertilizer, we added the finely ground mineral P fertilizer to the soils and homogenized soil and fertilizer thoroughly before the wheat was sown. The ¹⁰⁹Cd-labeled mineral P fertilizer was added to half of the pots. The other half received unlabeled mineral P fertilizer that was processed in the same manner, following the protocol outlined in section 2.2. Results for wheat that was grown on the same
soils without receiving fertilizer (control) were obtained for wheat grown in the same period and are already published in Wiggenhauser et al. (2016). For each soil and treatment, three (control, fertilizer with stable isotopes) or four (fertilizer with radio isotope) replicates were prepared. Only three replicates were prepared for treatments designated for stable isotope analyses because of the time-consuming sample preparation that is required for these measurements. For the control treatment of Oen, one of the four plants died (Wiggenhauser et al., 2016).

For safety reasons, we did not collect root samples from the $^{109}$Cd treatments. Based on previous findings (Jensen and Mosbæk, 1990), we expected that most of the $^{109}$Cd remains in the soil and roots. Thus, the removal of soil from roots would have produced a dust enriched in $^{109}$Cd, which has a long radioactive (462 d) and biological half-life (~10 y, Godt et al., 2006). Consequently, we harvested only straw and grains from the $^{109}$Cd-TSP treatment.

With the harvested plant parts, we were able to determine the Cd input to the food chain (grain), the total Cd export from the soil after harvest (shoot = straw + grain) and the Cd that could be in some cases returned to the soils with straw. After harvest, the plant samples were milled and digested with HNO$_3$ in a single reaction chamber microwave system (turboWave, MWS microwave systems, Wiggenhauser et al., 2016). Radioactive Cd samples were milled in a fume hood while wearing a protective mask.
2.4. Concentration and isotope measurements

Cadmium concentrations were determined using a quadrupole ICP-MS (Agilent 7500ce). The $^{109}$Cd activity was measured using the 88 keV photon emission line by means of an intrinsic high-resolution Ge gamma spectrometer equipped with the spectrum software Interwinner (version 7.1.). For the determination of stable isotope ratios, Cd was first separated and purified using a three-stage column chemistry procedure and the analyzed using a double-spike technique by multiple collector inductively coupled plasma mass spectrometry (MC-ICP-MS, Nu Plasma HR, Wiggenhauser et al., 2016).

2.5. Calculations and Statistics

The fraction of Cd in wheat derived from the fertilizer ($Cd_{diff}$) was calculated with Eq. 1:

$$ Cd_{diff} = \frac{SA_{wheat}}{SA_{fertilizer}} \times 100 \quad (1) $$

where SA denotes the specific activity [Bq (µg Cd)$^{-1}$] of the fertilizer or in a specific wheat part (straw, grain, shoot). The specific activity was calculated with Eq. 2:

$$ SA = \frac{^{109}Cd}{Cd} \quad (2) $$

where $^{109}$Cd denotes the radioactivity [Bq (g dry matter [DM]$^{-1}$)] of the straw, grain, or shoot and the denominator represents the Cd concentration [µg Cd (g DM)$^{-1}$] of the plant parts. Equations 1 and 2 can be used to calculate the Cd mass in straw and grains derived from the fertilizer with Eq. 3:
where \( Cd_{\text{mass.diff}} \) denotes the Cd mass [\( \mu g \) Cd (g DM plant\(^{-1}\))] in straw, grain, or shoot. Finally, knowing the fertilizer-derived Cd mass in these plant parts, the fertilizer recovery [%] can be calculated with Eq. 4:

\[
Cd_{\text{recovery}} = \frac{Cd_{\text{mass.diff}}}{Cd_{\text{mass.added}}} \times 100
\] (4)

where \( Cd_{\text{mass.added}} \) denotes the Cd mass [\( \mu g \) Cd (g DM soil\(^{-1}\])] introduced into the soil-wheat systems for the fertilizer application.

Stable isotope ratios were used to calculate \( Cd_{\text{diff}} \) [%] with an end member-mixing model in the soil-fertilizer-wheat systems of Oen (Eq. 5):

\[
Cd_{\text{diff.shoot}} = \left( \frac{\delta_{\text{shoot.ferti}} - \Delta_{\text{corr.shoot}}}{\delta_{\text{fertilizer}} - \delta_{\text{soil.ctrl}}} \right) \times 100
\] (5)

The calculated \( Cd_{\text{diff.shoot}} \) values can be further applied to Eq. 3 and 4. In Eq. 5, all \( \delta \) values refer to \( \delta^{114/110}\text{Cd} \) [%\text{o}], which was expressed relative to the NIST SRM 3108 isotope reference material (Abouchami et al., 2013). The \( \delta_{\text{fertilizer}} \) and \( \delta_{\text{shoot.ferti}} \) values denote the Cd isotope ratios of the mineral P fertilizer and the shoots that were grown with fertilizer added, respectively. To calculate \( Cd_{\text{diff}} \) for straw and grain, isotope ratios with the index “shoot” need to be replaced accordingly. \( \delta_{\text{soil.ctrl}} \) represents the Cd isotope ratio for the plant-available Cd in the control pot. In Oen, this soil pool is well represented by the \( \delta^{114/110}\text{Cd} \) value of the Ca(NO\(_3\))\(_2\) soil extract (Wiggenhauser et al., 2016). Furthermore, any
apparent Cd isotope fractionation within the soil-wheat system was corrected with Eq. 6:

\[ \Delta_{corr.\ shoot} = \delta_{\ shoot.\ ctrl} - \delta_{\ soil.\ ctrl} \]  \hspace{1cm} (6)

where \( \Delta_{corr.\ shoot} \) [%] denotes the apparent Cd isotope fractionation between the shoot and the soil \( \delta_{soil} \) that is represented by the Ca(NO\(_3\))\(_2\) soil extract. Eq. 6 can also be used to calculate the apparent isotope fractionation between shoot-root and grain-straw. The calculations thereby assume that the addition of mineral P fertilizer did not change the Cd isotope fractionation in the soil-wheat system. This assumption was tested by comparing the apparent isotope fractionation between the wheat shoot and the extractable Cd in the soil pool (\( \Delta^{114/110}Cd_{\ shoot-extractable} \)) and between the analyzed shoot parts for samples from both fertilized and control pots.

We also calculated the apparent fertilizer use efficiency (AUE, Muñoz et al., 2004) for Cd. The AUE [%] expresses the relative increase of the Cd mass in the wheat from the addition of Cd with the mineral P fertilizer (Eq. 7):

\[ AUE = \frac{Cd_{wheat.\ ferti} - Cd_{wheat.\ ctrl}}{Cd_{ferti}} \times 100 \]  \hspace{1cm} (7)

where \( Cd_{wheat.\ ferti} \) and \( Cd_{wheat.\ ctrl} \) denotes the Cd mass [µg (kg soil)\(^{-1}\)] in the shoot, straw, or grain of a fertilized (non labeled) or unfertilized control plant, respectively. \( Cd_{ferti} \) denotes the Cd mass [µg (kg soil)\(^{-1}\)] that was added to the pots with the mineral P fertilizer.

To test if the observed mean Cd concentrations, masses, \( \delta^{114/110}Cd \) values, \( Cd_{diff} \) and \( Cd_{recovery} \) were significantly different from each other, an ANOVA procedure
followed by a Tukey HSD post-hoc test were applied. If required, the response variable was log$_{10}$, square root, or 1/x transformed to produce homogeneity of variance and normal distribution of the residuals.
3. Results

3.1. Dry matter production and Cd concentrations in wheat shoots

Wheat shoot DM production differed among the soils (Table 1). Shoot DM production was significantly higher in Wied (15.8 to 16.4 g) compared to Oen (10.1 to 14.9 g) and LQ (13.2 to 15.8 g). Significantly less shoot DM was produced in the treatments that did not receive mineral P fertilizer (10.1 to 15.8 g) compared with the wheat that had received $^{109}$Cd-labeled mineral P fertilizer (14.9 to 18.4 g).

The different soil properties significantly influenced Cd concentrations of the wheat shoots (Table 1). Cadmium concentration in the shoots were higher in Oen (95 to 127 ng g$^{-1}$) than Wied (72 to 98 ng g$^{-1}$) and higher in Wied than in LQ (37 to 59 ng g$^{-1}$). The fertilizer addition had no significant impact on Cd concentrations in the shoot, straw and grain, except in Oen, where the Cd concentration was higher in plants treated with labeled or unlabeled P fertilizer (127 to 144 ng g$^{-1}$ for shoots) than for the control treatment (95 ng g$^{-1}$).

Furthermore, the accumulated Cd mass in the shoot varied strongly among the soils (average: LQ 0.63 ± 0.08 µg, Oen 1.61 ± 0.56 µg, Wied 1.38 ± 0.22 µg, Table 1) but was less influenced by the mineral P fertilizer addition (average: control 0.96 ± 0.22 µg, mineral P fertilizer 1.13 ± 0.63 µg).
<table>
<thead>
<tr>
<th>Treatment</th>
<th>DM</th>
<th>Cd concentration</th>
<th>Cd mass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LQ'</td>
<td>Own'</td>
<td>Wied'</td>
</tr>
<tr>
<td></td>
<td>mean sd²</td>
<td>mean sd²</td>
<td>mean sd²</td>
</tr>
<tr>
<td>Control</td>
<td>13.2 ± 4.38 ab</td>
<td>10.1 ± 3.77 b</td>
<td>15.8 ± 9.1 cd</td>
</tr>
<tr>
<td>Shoot 1³⁵⁰Cd P fertilizer</td>
<td>15.8 ± 1.55 ab</td>
<td>14.9 ± 1.05 ab</td>
<td>18.4 ± 0.46 a</td>
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<td>Shoot P fertilizer</td>
<td>13.4 ± 3.66 ab</td>
<td>13.1 ± 1.22 ab</td>
<td>16.4 ± 0.89 a</td>
</tr>
<tr>
<td>Control</td>
<td>7.34 ± 2.53 ab</td>
<td>5.98 ± 2.32 b</td>
<td>8.39 ± 0.43 a</td>
</tr>
<tr>
<td>Grain 1³⁵⁰Cd P fertilizer</td>
<td>9.38 ± 0.58 ab</td>
<td>9.49 ± 0.56 ab</td>
<td>10.3 ± 0.55 a</td>
</tr>
<tr>
<td>Grain P fertilizer</td>
<td>7.65 ± 2.07 ab</td>
<td>7.88 ± 0.83 ab</td>
<td>9.15 ± 0.59 a</td>
</tr>
<tr>
<td>Control</td>
<td>5.84 ± 1.81 ac</td>
<td>4.11 ± 1.46 c</td>
<td>7.42 ± 0.85 ab</td>
</tr>
<tr>
<td>Straw 1³⁵⁰Cd P fertilizer</td>
<td>6.38 ± 0.81 ab</td>
<td>5.39 ± 0.59 bc</td>
<td>8.10 ± 0.69 a</td>
</tr>
<tr>
<td>Straw P fertilizer</td>
<td>5.73 ± 1.60 ab</td>
<td>5.26 ± 1.14 bc</td>
<td>7.21 ± 0.80 ab</td>
</tr>
</tbody>
</table>

*P-fertilizer and 1³⁵⁰Cd P-fertilizer refers to the stable and radio isotope approaches, respectively. 1 and 6 refer to Cd recovery values that were calculated with and without outlier, respectively (see text for details). The data from control treatment was taken from Wiegnerhouser et al. 2016.

²sd denotes standard deviation of n = 5 (stable isotope, AUE) and n = 4 samples (radio isotope). Small letters denote significant differences between the mean determined with a two way ANOVA (* soil1 * fertilizer2) and Tukey HSD post hoc test.

*1Q, 2Q, and Wied refer to the origin of the arable soils used for the pot trial, i.e. the Swiss villages of landfill, Oerlikon and Wiedlisbach, respectively.
3.2. **Source tracing of Cd using the P fertilizer labeled with \(^{109}\text{Cd}\)**

The proportion of Cd in straw and grains of wheat that derived from the mineral P fertilizer labeled with \(^{109}\text{Cd}\) (Cd\(_{\text{dff}}\), Eq.1) differed significantly among the soils (Figure 1a). The Cd\(_{\text{dff}}\) values were highest in Wied (14.9 ± 0.73 %), followed by LQ (8.1 ± 1.31 %) and Oen (6.5 ± 0.23 %). Furthermore, Cd\(_{\text{dff}}\) was higher in straw (7.9 to 16.7 %) than in the grains (4.7 to 10.6 %) for all soils (Figure 1a).

From the total Cd introduced into the soil-wheat systems by the mineral P fertilizer, only a minor part (<2.2 %) was recovered in straw and grains of wheat (Figure 1b). Among the soils, most Cd was recovered in the shoots in Wied (2.2 ± 0.10 %), followed by Oen (1.5 ± 0.03 %) and LQ (0.50 ± 0.05 %, Figure 1b). Among the shoot organs, less Cd was recovered in the grains (0.12 to 0.47 %) compared to the straw (0.40 to 1.7 %) on all soils.
3.3. Source tracing of Cd applied with mineral P fertilizer using the stable isotopes and mass balance

The fertilizer addition altered the apparent isotope fractionation of Cd between the whole plant and the Ca(NO₃)₂ extractable soil pool in Oen. Specifically, the fractionation $\Delta^{114/110}\text{Cd}_{\text{wholeplant-extractable}}$ was $-0.04 \pm 0.07 \%$ for the control plants and $-0.28 \pm 0.07 \%$ for the plants treated with the P fertilizer (Figure 2a). However, the Cd extractable soil pools had identical isotope compositions within error, with results of $\delta^{114/110}\text{Cd}_{\text{control}} = 0.50 \pm 0.01 \%$ (Wiggenhauser et al.,

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Figure 1: a) Fraction of Cd in straw and grains of wheat that was derived from the fertilizer ($\text{Cd}_{\text{dff}}$) calculated with radio isotopes. b) Fraction of Cd introduced with the fertilizer that was recovered in straw and grains of wheat ($\text{Cd}_{\text{recovery}}$). Brown circles and green triangles denote grain and straw, respectively. LQ, Oen, and Wied denote the arable soils used. Error bars denote standard deviation of $n = 4$ replicates. Small letters denote significant differences between the means of straw and grains of all soils (two-way ANOVA and Tukey HSD post-hoc test)
2016) and $\delta^{114/110}\text{Cd}_{\text{fertilizer}} = 0.56 \pm 0.06$‰ (Table A1). The $\delta^{114/110}\text{Cd}$ values of shoots, straw and grains from the control and fertilizer treatments also did not differ significantly from each other (Figure 2b). However, wheat shoots, as well as grains and straw, appear to have slightly lower $\delta^{114/110}\text{Cd}$ for the fertilizer (0.37 to 0.40 ‰) than the control treatment (0.52 to 0.62 ‰, Figure 2b). Whilst this difference was not significant, it markedly influenced the results of the Cd$_{\text{diff}}$ calculations that were based on the stable isotope mass balance (E1. 5; see details in section 4.1).

Figure 2: Stable isotope results obtained for soil-wheat systems from Oen (no results are available for the other locations). a) Apparent Cd isotope fractionation in different compartments of soil-wheat systems. b) Cd isotope ratios of straw and grains of soil-fertilizer-wheat systems. Different shapes and colors of the symbols denotes soil-wheat systems that received (green triangles) or did not receive mineral P fertilizer (blue circles). Error bars denote standard deviation of $n = 3$ replicates. Small letters denote significant differences between the paired means. Plant- available Cd was extracted from the bulk soil using Ca(NO$_3$)$_2$. The Oen control data were taken from Wiggenhauser et al. 2016.
Source tracing calculations that employ stable isotopes revealed that 36 ± 5 %
of the Cd in the Oen wheat shoots were derived from the fertilizer (Figure 3). 
To test the sensitivity of the Cd_{diff} calculation using stable isotopes (Eq. 5), we 
recalculated the mean Δ_{corr.shoot} (Eq. 6) following removal of the outlier in the 
control treatment, which was strongly enriched in heavy isotopes compared to 
the other treatments due to the low DM production and, as a consequence, 
limited Cd uptake from the soil (Table 1). The recalculated results, illustrated in 
Figure 3 as “stable isotopes II”, feature lower Cd_{diff} values (22 to 24 %) than the 
original ‘stable isotope I” data. Source tracing based on the natural stable Cd 
isotope composition of the system hence resulted in significantly higher Cd_{diff} 
values (22 to 30 %) than source tracing calculations for the radio label. As a 
consequence, the stable isotope data suggest a significantly higher recovery of 
the fertilizer-derived Cd in the wheat (Cd_{recovery}) than the radio isotope results 
(Table 2).

In addition, the Cd recovery was also calculated based on the AUE, which is a 
completely non-isotope mass balance approach (Eq. 7; Table 2). Calculations 
with the AUE method produce significantly higher Cd recoveries for the shoots 
(3.16 to 6.71 %) than the radio isotope approach (1.46 ± 0.06 %) and are also 
somewhat lower than the stable isotope results (4.31 to 7.37 %). Importantly, 
the standard deviations of the stable isotope and AUE Cd_{recovery} data (sd = 0.84 
to 3.77 %) are more than one order of magnitude larger compared to the radio 
isotope results, which have sd values of 0.06 to 0.09 % (Table 2).
Table 2: Comparison of the Cd recovery from freshly applied mineral P fertilizer calculated with radio isotopes, stable isotopes and mass balance (Apparent Use Efficiency, AUE)

<table>
<thead>
<tr>
<th>Method</th>
<th>Shoot recovery (%)</th>
<th>Straw recovery (%)</th>
<th>Grain recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radio isotope</td>
<td>1.46 ± 0.06 b</td>
<td>0.99 ± 0.07 b</td>
<td>0.47 ± 0.09 b</td>
</tr>
<tr>
<td>Stable isotope I</td>
<td>7.37 ± 3.77 a</td>
<td>4.24 ± 2.43 ab</td>
<td>3.13 ± 1.33 a</td>
</tr>
<tr>
<td>Stable isotope II</td>
<td>4.31 ± 3.05 ab</td>
<td>3.00 ± 2.11 ab</td>
<td>1.31 ± 0.94 ab</td>
</tr>
<tr>
<td>AUE I</td>
<td>6.71 ± 2.39 a</td>
<td>5.09 ± 2.01 a</td>
<td>1.62 ± 0.84 ab</td>
</tr>
<tr>
<td>AUE II</td>
<td>3.16 ± 2.38 ab</td>
<td>2.78 ± 2.01 ab</td>
<td>0.38 ± 0.84 b</td>
</tr>
</tbody>
</table>

Figure 3: Comparison of methods to calculate the fraction of Cd derived from the mineral P fertilizer in shoot, straw and grains of wheat (Cd_{dff}) grown on soils from Oen (no results are available for the other locations). Different shapes and colors of the symbols denote different calculation methods: red circles = stable isotopes I, light green triangles = stable isotope II, dark green rectangles = radio isotopes. The stable isotope I and II were calculated either including or excluding a single outlier in the data reduction, respectively (see text for details). Error bars denote standard deviation of n = 3 replicates. Small letters denote significant differences of the mean for each box. The Oen control data that were used to calculate the stable isotope based Cd_{dff} values are from Wiggenhauser et al. (2016).

Table 2: Comparison of the Cd recovery from freshly applied mineral P fertilizer calculated with radio isotopes, stable isotopes and mass balance (Apparent Use Efficiency, AUE)

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<thead>
<tr>
<th>Method</th>
<th>Shoot recovery (%)</th>
<th>Straw recovery (%)</th>
<th>Grain recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radio isotope</td>
<td>1.46 ± 0.06 b</td>
<td>0.99 ± 0.07 b</td>
<td>0.47 ± 0.09 b</td>
</tr>
<tr>
<td>Stable isotope I</td>
<td>7.37 ± 3.77 a</td>
<td>4.24 ± 2.43 ab</td>
<td>3.13 ± 1.33 a</td>
</tr>
<tr>
<td>Stable isotope II</td>
<td>4.31 ± 3.05 ab</td>
<td>3.00 ± 2.11 ab</td>
<td>1.31 ± 0.94 ab</td>
</tr>
<tr>
<td>AUE I</td>
<td>6.71 ± 2.39 a</td>
<td>5.09 ± 2.01 a</td>
<td>1.62 ± 0.84 ab</td>
</tr>
<tr>
<td>AUE II</td>
<td>3.16 ± 2.38 ab</td>
<td>2.78 ± 2.01 ab</td>
<td>0.38 ± 0.84 b</td>
</tr>
</tbody>
</table>

*1 and II refer to Cd recoveries that were calculated with and without outlier, respectively (see text for details).

*sd denotes standard deviation of n = 3 (stable isotope, AUE control) and n = 4 samples (radio isotopes, AUE fertilized). Small letters denote significant differences between the mean within each column.
4. Discussion

4.1. Comparison of Cd source tracing methods

The $\text{Cd}_{\text{diff}}$ and the $\text{Cd}_{\text{recovery}}$ values that were determined for wheat based on stable isotope data or a mass balance (AUE) approach are significantly higher compared to the radio isotope results, which were obtained using a mineral P fertilizer labeled with $^{109}\text{Cd}$ (Figure 3).

For the stable isotope calculations, the removal of the outlier from the control treatment data produced markedly lower $\text{Cd}_{\text{diff}}$ values, by up to -26% (Figure 3). This demonstrates that even small changes or uncertainties in the isotope compositions of the sources and sinks have a significant impact on the calculated $\text{Cd}_{\text{diff}}$ results. Furthermore, the $\delta^{114/110}\text{Cd}$ values of the sources range from 0.02 to 0.50 ‰. For this range, a 1% contribution of P fertilizer-derived Cd to the shoot changes the isotope composition of the shoot by only 0.005 ‰.

In comparison, the external reproducibility of the isotope measurements was $\pm 0.083$ ‰ (2sd, Wiggenhauser et al., 2016), which is equivalent to an uncertainty of about $\pm 16$ % for the final $\text{Cd}_{\text{diff}}$ values. As such, our data do not fulfill the prerequisite that the isotope compositions of the two Cd sources for the plants (mineral P fertilizer and soil) are sufficiently distinct to enable precise source tracing (Wiederhold, 2015).

In addition, any change of the apparent isotope fractionation between soil and wheat also influences the source tracing results. The application of mineral P fertilizer did not significantly change the isotope composition of the $\text{Ca(NO}_3\text{)}_2$ extract (considering the analytical uncertainty; Table A1), but it did alter the
isotope fractionation between the extract and the shoot. This suggests that the
mineral P fertilizer, comprised mainly of $\text{Ca}^{2+}$, $\text{PO}_4^{3-}$ and $\text{SO}_4^{2-}$ ions (Fig. A1),
might have altered the speciation of the extractable and plant-available Cd,
thereby also affecting the isotope compositions of the Cd pool that was
transferred to the wheat shoot. Consequently, the shift in $\delta^{114/110}\text{Cd}$ for the wheat
shoots was partly due to stable isotope fractionation and not exclusively a
consequence of mixing Cd derived from the two relevant sources.

The addition of a radio isotope to a mineral P fertilizer can potentially create
artifacts. For example, labeling of a fertilizer with an isotope requires that the
introduced radio isotopes are homogeneously distributed (Frossard et al., 2011).
In this study, a qualitative XRD analysis showed that the labeling process did
not alter the Ca speciation during the labeling procedure (Fig. A1). Furthermore, the water- soluble Cd fraction of the mineral P fertilizer (~ 40 %)
was not changed by the labeling procedure (Table A2). Thus, it is therefore
likely that the introduced, carrier-free $^{109}\text{Cd}$ could readily formed inorganic
complexes with $\text{PO}_4^{3-}$ and / or replaced native Cd, Ca or other metal cations that
were present in the mineral P fertilizer and in particular, its water-soluble pool.
Furthermore, Prochnow et al. (2008) showed that a part of the Ca present in
mineral P fertilizers is still hosted in weakly soluble minerals, such as apatite,
which accounted for about 3.1 to 6.4 % of the fertilizer mass. This suggests that
the radio labeling procedure used here might not have accessed and exchanged
with insoluble Cd pools within the fertilizers. As worst case scenario, the added
$^{109}\text{Cd}$ would have only exchanged with the water soluble pool which represents
41.3 % of the total Cd of the P-fertilizer (Table A2). If this scenario is applied to Eq. 1-4, Cd\textsubscript{recovery} values decrease from initially 0.50 to 2.2 % to 0.21 to 0.90 %.

Notably, such corrections would further increase the difference between the results of the radio isotope and the stable isotope approach.

The AUE mass balance approach to determine the Cd recovery in wheat shoots also produced higher Cd recovery values and larger standard deviations than the radio isotope approach (Table 2). The high Cd\textsubscript{recovery} results might reflect the small observed differences in the Cd masses of the control and fertilized wheat shoots, which significantly impacts the precision of the AUE method. The AUE mass balance approach is therefore less suitable for systems with low recoveries and more precise for nutrients with high recovery rates such as N and P (e.g., recovery higher than 30% for mineral fertilizer in similar soil-plant systems; Bosshard et al., 2009; Nanzer et al., 2014)

In summary, among the three approaches applied in this study, the radio isotope approach provides most robust results and these are applied in the following to constrain and discuss the fate of fertilizer-derived Cd in soil-wheat systems.

4.2. The fate of freshly applied Cd through P-fertilization

The Cd mass in the shoot is equal to the Cd mass exported from the soil during harvesting, if no straw is left at the soil surface (Figure 4). The export of Cd from soil to shoot varied significantly among the soils (Oen and Wied > LQ) and was lowest for the soil with the highest pH (Figure 4). This finding is in accordance with the results of previous studies and reflects that the solubility of
Cd increases with decreasing pH (Gray et al., 1999; Guttormsen et al., 1995) through proton-Cd exchange and less specific sorption (Loganathan et al., 2012). The export of Cd from soil to shoot was less influenced by the addition of mineral P fertilizer (Table 1), although the dose and Cd concentration of the added material represents a worst-case scenario for Cd input via fertilizer into Swiss agricultural systems (FOAG, 2015; Spiess, 2011). The strong influence of soil properties on Cd export into the shoot is in agreement with previous observations of a field study at the same sites (Imseng et al., 2018). However, the observed difference for the exported Cd between Oen and Wied was larger in the field study (Oen 2.6 times higher than Wied) than in the current pot study (1.5). The larger difference that observed in the former might reflect that Cd losses with seepage water were larger in the sandy soils of Wied (0.99 g h\(^{-1}\) y\(^{-1}\)) than in the clay rich Oen soils (0.02 g h\(^{-1}\) y\(^{-1}\), Imseng et al., 2018). Such losses did not occur in the pot trial of this investigation, as this featured a completely closed soil-fertilizer-wheat system, with no Cd losses or additions after sowing.
Although the addition of mineral P fertilizer generally did not increase the export of Cd from soil to wheat, up to 17 % and 11 % of the Cd in the straw and grains was derived from the fertilizer, respectively (Figure 1a). Moreover, Cd\text{diff} differed significantly between the soils whereby higher Cd\text{diff} was observed if more Cd was added to the soil, relative to the total soil Cd stock already present (Figure 4), in accord with previous results for soil-fertilizer-lettuce systems.
This suggests that the fertilizer-derived Cd was diluted by Cd already present in the soils.

The comparatively low Cd recoveries found here are, however, in good agreement with Cd recoveries determined for soil-fertilizer-lettuce systems (4 to 16%, Jensen and Mosbæk, 1990). The low recoveries suggest that the continuous applications of mineral P fertilizers over the past decades probably led to the accumulation of residual Cd. Field data obtained for arable soils of Switzerland supports this hypothesis. In particular, two recent studies attributed Cd accumulation in top- compared to subsoils at arable farming sites at least in part to the past use of mineral P fertilizer (Bigalke et al., 2016; Imseng et al., 2018). The build-up of a residual Cd pool might thereby have started as early as in the 1950s (Spiess, 2011). From that time until now, P-fertilization in Switzerland peaked in the 1970s and was then reduced by 80% until 2005. Thus, it is likely that the rate at which Cd was added by mineral P fertilizers was higher in the past, provided that the Cd concentrations of the fertilizers were similar in the past and present (FOAG, 2015). Furthermore, a residual Cd pool would not only change the total Cd stock of the soils, but also increase the total concentration of plant-available Cd (Gray et al., 2016; Hamon et al., 1998). As such, the residual mineral P fertilizer derived Cd pool would impact the transfer of Cd from soils to plant.

To constrain the size and the impact of a possible residual fertilizer-derived Cd pool in soils on current fluxes of Cd in soil-plant systems, it is desirable to conduct source tracing study of fertilizer-derived Cd for soils that differ only in
their fertilization history. As it was previously demonstrated for P, such an investigation can distinguish between contributions of geogenic soil-derived Cd, residual Cd in soil and Cd from mineral P fertilizer (Gallet et al., 2003). Such a study would thus help to improve the relationships between past P fertilizer applications and current Cd fluxes in arable soil systems.

5. Conclusion

Radio isotope methods provided the most robust approach for tracing Cd that was applied with mineral P fertilizer to soil-wheat systems. The application of natural Cd stable isotope data for this purpose provided estimates of the Cd that derived from the fertilizer and the Cd recovery in the what that were imprecise and too high because the isotope compositions of the two Cd sources (soil and fertilizer) were too similar. Cadmium source tracing using radioactive $^{109}$Cd revealed that only a small fraction ($< 3\%$) of the Cd that was applied with mineral P fertilizer reached the wheat shoots. The majority of the Cd that was added to soils with mineral P fertilizer hence remained in the soil and might contribute to the build-up of a residual Cd pool. We hypothesize that the past build-up of such a residual Cd pool in soils may significantly impact the current fluxes of Cd in soil-plant systems.

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**Literature**


