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Cadmium transfer in contaminated soil-rice systems: Insights from solid-state speciation analysis and stable isotope fractionation^{*}



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ABSTRACT

Initial Cadmium (Cd) isotope fractionation studies in cereals ascribed the retention of Cd and its light isotopes to the binding of Cd to sulfur (S). To better understand the relation of Cd binding to S and Cd isotope fractionation in soils and plants, we combined isotope and XAS speciation analyses in soil-rice systems that were rich in Cd and S. The systems included distinct water management (flooded vs. non-flooded) and rice accessions with (excluder) and without (non-excluder) functional membrane transporter OsHMA3 that transports Cd into root vacuoles. Initially, 13% of Cd in the soil was bound to S. Through soil flooding, the proportion of Cd bound to S increased to 100%. Soil flooding enriched the rice plants towards heavy isotopes ($\delta^{114/110}\text{Cd} = -0.37$ to -0.39%) compared to the plants that grew on non-flooded soils ($\delta^{114/110}\text{Cd} = -0.45$ to -0.56%) suggesting that preferentially light Cd isotopes precipitated into Cd sulfides. Isotope compositions in CaCl_2 root extracts indicated that the root surface contributed to the isotope shift between soil and plant during soil flooding. In rice roots, Cd was fully bound to S in all treatments. The roots in the excluder rice strongly retained Cd and its light isotopes while heavy isotopes were transported to the shoots ($\Delta^{114/110}\text{Cd}_{\text{shoot-root}} = 0.16$ – 0.19%). The non-excluder rice accumulated Cd in shoots and the apparent difference in isotope composition between roots and shoots was smaller than that of the excluder rice ($\Delta^{114/110}\text{Cd}_{\text{shoot-root}} = -0.02$ to 0.08%). We ascribe the retention of light Cd isotopes in the roots of the excluder rice to the membrane transport of Cd by OsHMA3 and/or chelating Cd–S complexes in the vacuole. Cd–S was the major binding form in flooded soils and rice roots and partly contributed to the immobilization of Cd and its light isotopes in soil-rice systems.

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1. Introduction

Cadmium (Cd) is a harmful element for humans due to its toxicity and long half-life time in our bodies (Fransson et al., 2014; Godt et al., 2006). To lower the risk of Cd exposure, its concentrations need to be minimized in cereals, which are major sources of

Cd for humans (Filippini et al., 2018; Kim et al., 2018; Vromman et al., 2010). Rice, a staple food in several Asian countries, is a particularly high source of Cd for humans (Chunhabundit, 2016; Meharg et al., 2013; Song et al., 2017). To reduce Cd concentrations in rice, the mechanisms that determine the Cd availability from soil to plants, its plant uptake and its transport processes need to be well understood.

There is potential for using metal isotopes as a tool for tracing processes in biochemical systems (Wiederhold, 2015), including complex soil-plant systems (Caldelas and Weiss, 2017). First Cd isotope process tracing studies in soil-plant systems exhibited consistent patterns of Cd isotope fractionation. For instance, light Cd isotopes were preferentially taken up by plants (Imseng et al.,

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2019; Moore et al., 2020; Wei et al., 2016). Within cereal plants, a successive enrichment of heavy isotopes occurred during the transport of Cd from the roots to the grains of cereals (Imseng et al., 2019; Wiggengauser et al., 2016). In non-cereal plants, Cd isotopes were less systematically fractionated (Barraza et al., 2019; Wei et al., 2016), except for cacao seedlings in which shoots were enriched in heavy isotopes compared to roots (Moore et al., 2020). Furthermore, Cd isotopes in the soil solution were consistently enriched in heavy isotopes compared to the bulk soil (Imseng et al., 2019; Wiggengauser et al., 2016; Zhang et al., 2016). These initial studies generated tentative conceptual models to explain Cd isotope fractionation in soil-plant systems (Imseng et al., 2019). One of the major findings so far, the enrichment of heavy isotopes in grains of cereals, was explained by Cd binding to thiol groups (R-SH) that retain light isotopes in roots and shoots (Wiggengauser et al., 2018). This explanation was built on the fact that Cd has a marked affinity for thiols (Sóvágó and Várnagy, 2013) and the binding of Cd to S donor ligands favors light Cd isotopes compared to O and N donors (Guinoiseau et al., 2018; Yang et al., 2015; Zhao et al. in revision). The preferential sorption of light Cd isotopes to S may be induced by the low electronegativity of S and the significantly longer bond lengths to S than to O and N donors (Albarede et al., 2016; Imseng et al., 2019). Thus, Cd isotopes could potentially serve as marker for Cd binding to S in plants and in soils where S in soil organic matter (SOM) or sulfides can be an important binding site (Fulda et al., 2013; Karlsson et al., 2005, 2007).

In the past few years, several membrane proteins that partly control the mobility Cd within rice have been identified (W. Wang et al., 2019). These advances generated a variety of genetically well defined rice cultivars with distinct Cd accumulation properties. For instance, the tonoplast-localized transporter OsHMA3 controls the transport of Cd into root vacuoles (Shao et al., 2017; Ueno et al., 2010). Rice accessions with functional and non-functional OsHMA3 respectively have less or more Cd transport from root to shoot. Such rice accessions could be employed to better understand the role of vacuolar sequestration and Cd speciation regarding Cd retention in roots (Yan et al., 2016). Furthermore, rice is mainly cultivated in flooded soils which induces changes in the soil redox state (Fulda et al., 2013; Furuya et al., 2016; J. Wang et al., 2019). These changes can determine the Cd concentration in soil solutions due to e.g. pH changes and the formation of insoluble sulfides. Therefore, soil-rice systems are an ideal model to study Cd isotope fractionation soil-plant systems.

In this study, we aimed to extend the scope of using Cd stable isotopes for process tracing by revealing the relation between Cd isotope composition and Cd speciation in soil-rice systems. To this end, we analyzed Cd isotope compositions and Cd speciation in contrasting and well defined soil-rice systems. Specifically, we grew rice (*Oryza sativa* L.) with functional and non-functional OsHMA3 vacuolar transporters in pots under flooded and non-flooded soil conditions. To probe Cd speciation in soils and plants, we employed synchrotron X-ray absorption spectroscopy (XAS), as XAS analysis can identify inorganic and organic forms of Cd bound to both S- and O-functional groups in soils. Finally, we evaluated the potential of Cd isotope process tracing to advance the knowledge of the mechanisms that control the mobility of Cd in soil-rice systems.

2. Material and methods

2.1. Rice growth experiment

A cross-factorial pot study was conducted using the factors 'rice accession' (two treatments: excluder, non-excluder) and 'water management' (two treatments: non-flooded soil (upland

conditions), flooded soil (lowland conditions). Table S1 provides a chronological overview of the experiment.

A 1.5 kg portion of dry rice paddy soil (5 mm sieved, Table S2) was spiked with Cd(NO₃)₂ to obtain a bulk soil Cd concentration of 15 mg Cd kg⁻¹ (soil dry weight). After 21 days of aging, each pot was fertilized (Table S3) and equipped with a suction cup for soil solution sampling (Rhizon, SDEC France). Chapter 1 of the supporting information (SI.1) provides more information.

Two rice (*Oryza sativa* L.) accessions were cultivated with functional (cv- Taichung-65 (T65), 'excluder') or non-functional (TCM-213, 'non-excluder') root vacuolar Cd OsHMA3 transporters (Shao et al., 2017). Plants were cultivated in a growth chamber (Fotoclimat, France), Table S4 provides detailed settings. After the germination and growth of rice in hydroponic cultures, the plants were transplanted into the distinct soils (25 days after sowing, DAS). By this time, the plants have started to develop tiller 2 and 3. During plant growth, the water level of the non-flooded soils was kept between 60 and 100% water holding capacity using non-degassed water. This is equal to a dry soil to water ratio of 1.6–2.7. The 'flooded soils' were kept at a soil:water ratio of 0.4–0.6. This ratio resulted in a 6 cm water column above the soil, which ensured that the soil surface was permanently covered with water (details in SI.1).

2.2. Plant and soil sampling

The soil, root and shoot of each treatment was sampled when three tillers were carrying flowers (Table S1). This growth stage was chosen since it can be clearly identified. In addition, we planned to compare in the course of a different experiment the isotope composition of rice plants at flowering and maturity. To harvest the shoots, they were cut <1 cm above the soil surface, rinsed using deionized water, dried using a paper towel, and weighed. The roots were gently separated from the soil at atmospheric conditions (non-flooded soil) or in a glove bag filled with N₂. The roots were then further treated at atmospheric conditions. The soil that stuck to the roots was removed by dipping the roots in deionized water that immediately turned into a soil suspension and then gently dried using paper towels. Cadmium that was sorbed to root cell walls was extracted from the roots using 10 mM CaCl₂ (Straczek et al., 2008; Talke et al., 2006). After the roots were separated from the soil, the soils were sampled. In the case of the non-flooded soil, about 20 g of soil was collected and dried in the oven at 55 °C under atmospheric conditions. The flooded soils were sampled in glove bags (N₂ atmosphere), transferred into a falcon tube, closed and sealed, and then transferred into a glove box (N₂ atmosphere). The samples were then kept in a desiccator, dried in a vacuum for 2 days (Stetten et al., 2018), and kept there until the samples were prepared for solid state speciation measurements in liquid N. Details in SI.3.

We sampled the soil solution at different time points to trace the dynamics of Cd and its isotopes during soil flooding. The soil solution was sampled using suction cups (Rhizon, SDEC France). We monitored pH, Eh, and Fe(II) weekly. Dissolved organic carbon, Cd concentration, Cd isotopes, and the concentrations of major anions and cations of these samples were analyzed at a later stage. Soil solution data were then used to model Cd speciation in the soil solution using visual MINTEQ (Groenenberg et al., 2010; Imseng et al., 2019; Pribyl, 2010; Ren et al., 2015). For isotope analysis, the different sampling time points were merged into periods in order to yield sufficient Cd for isotope analysis (details in SI.2).

2.3. Sample processing, concentration, and isotope analysis

All samples were digested on hotplates at 120 °C using HNO₃,

HCl, HF, and H₂O₂. Prior to the isotope analysis, matrix elements were removed from the samples using a slightly adapted one stage anion exchange chromatography procedure that was developed for samples in Cd contaminated environments (Table S5, Cloquet et al., 2005; Wei et al., 2015). In short, matrix elements (e.g. Ca, Zn, Sn) were removed from the sample using different concentrations of HCl while Cd was eluted from the column using 0.0012M HCl. This purification procedure resulted in a Cd recovery ranging from 90 to 102% (mean = 97%). The procedural blank was <0.12% in all cases and Cd:Sn mass ratios were >1000 in the purified samples. Neither recovery, blank nor Sn interference significantly altered the isotope compositions in the samples.

Cd isotope compositions were measured using a high-resolution multicollector ICP mass spectrometer (MC-ICPMS, Neptune Plus, Thermo Scientific) in 'dry' plasma conditions (Cloquet et al., 2005; Pallavicini et al., 2014; Wombacher et al., 2003). To correct for instrumental mass bias, 'standard-sample bracketing' was applied and isotope standards and samples were doped with silver. Frequent measurements of internal and external standard samples revealed robust results for Cd concentration and Cd isotope measurements (Table S6). Details in SI.4.

2.4. Cadmium speciation through X-ray absorption spectroscopy (XAS)

Once the soil and plants were sampled, the samples were milled in liquid N using a cryo-grinder (Pulverisette23, Fritsch) and then pressed into pellets in cryo conditions. Samples were either kept at -80 °C or in liquid N until the speciation analysis took place in order to avoid changes in Cd speciation. Cadmium K-edge XAS measurements were mostly conducted at SAMBA beamline (SOLEIL synchrotron, France). The X-ray Absorption Near Edge Structure (XANES) part, and, when possible, the Extended X-ray Absorption Fine Structure (EXAFS) part of the spectra were analyzed. All samples were recorded at 20 K using a helium cryostat in fluorescence mode. Details can be found in SI.5.

After normalization, the XANES and when possible the EXAFS spectra were treated by linear combination fits (LCFs) using XAS software (Athena, Demeter, Ravel and Newville, 2005) that employed a database of Cd reference spectra (Fulda et al., 2013; Huguet et al., 2012, 2015). Up to three standard spectra were used for the LCFs that were then grouped as 'Cd-O' (Cd bound to COOH/OH groups of organic matter or Cd sorbed to minerals), 'Cd-S' which represents 'Cd thiols or mixed/amorphous CdS' (i.e., Cd bound to cysteine, glutathione, phytochelatin, mixed Cd/Zn/Fe sulfide (Huguet et al., 2015) or weakly crystallized CdS), and 'crystalline CdS' (greenockite). In addition, soil spectra Cd structural parameters were determined by shell fittings using ARTEMIS software (Ravel and Newville, 2005). Table S7 and Figure S1 summarize all XAS data.

2.5. Calculations

Isotope ratios of ¹¹⁴Cd/¹¹⁰Cd of samples are expressed relative to the ¹¹⁴Cd/¹¹⁰Cd isotope ratio of isotope reference material NIST₃₁₀₈ as δ in permill,

$$\delta^{114/110}\text{Cd} = \left[\frac{\left(\frac{^{114}\text{Cd}}{^{110}\text{Cd}} \right)_{\text{sample}}}{\left(\frac{^{114}\text{Cd}}{^{110}\text{Cd}} \right)_{\text{NIST.3108}}} - 1 \right] \times 1000 \quad (1)$$

The Cd isotope composition of the entire plant (root + shoot) was calculated as a weighted arithmetic mean,

$$\delta^{114/110}\text{Cd}_{\text{wholeplant}} = \left(f_{\text{root}} \cdot \delta^{114/110}\text{Cd}_{\text{root}} \right) + \left(f_{\text{shoot}} \cdot \delta^{114/110}\text{Cd}_{\text{shoot}} \right) \quad (2)$$

where 'f' denotes the Cd mass fraction of the root or shoot relative to the total Cd mass in the plant.

The apparent difference in Cd isotope compositions 'Δ' was expressed by subtracting δ^{114/110}Cd values from each other,

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

where 'A' and 'B' are the δ^{114/110}Cd values of the compartments of the system, e.g. shoot and root.

2.6. Statistics

Significant differences in the mean between the treatments of n = 4 experimental replicates (i.e. rice that grew individually in a pot) were determined using ANOVA followed by a Tukey HSD test or a non-parametric Kruskal Wallis test. For bulk soil and soil solution samples, less than n = 4 experimental replicates were analyzed. For some samples, it was not possible to sample or analyze n = 4 experimental replicates, thus, no statistical tests were applied. For the bulk soil, the uncertainty of individually processed replicates was expressed as 2sd of n = 2 processing replicates. In the case of the temporal changes to the isotope compositions of the soil solution, samples were merged to yield sufficient Cd for isotope analysis (SI.2). Error bars were expressed as sd of n = 2 experimental replicates. XAS measurements could not be applied for each experimental replicate due to limited synchrotron beamtime. Based on our replicate measurements and as a rule of thumb, the precision of the linear combination fits (LCFs) is set to 10% (Castillo-Michel et al., 2017). Consequently, we considered Cd speciation to be significantly different from each other when the results differed by 20%. Details in SI.6.

3. Results and discussion

3.1. General characterization of the soil-rice systems

Prior to the pot trial, the soil was spiked with Cd that it contained 15 mg Cd kg⁻¹, thereby ensuring that Cd XANES spectra could be acquired in the soil-rice system. This concentration is in the upper range of Cd contamination when compared to Cd contaminated arable soils and mainly appears in areas of mining and smelting activities (Liu et al., 2016; Sriprachote et al., 2012; Zhang et al., 2015). Additionally, the soil was characterized as having a sandy texture and a S content (520 mg (kg soil)⁻¹ including fertilization) that was in the upper range when compared to other paddy soils (20–817 mg (kg soil)⁻¹, Neue and Marmaril, 1985). The S concentrations in the rice shoots were high (1.77–6.24 mg g⁻¹, Table S8) which indicates that S in the soil was readily bioavailable (Dobermann and Fairhurst, 2000).

The Cd concentrations in the shoots of the non-excluder rice were 7–12 times higher than in the excluder rice (Table 1). The high Cd concentrations in the non-excluder shoots (64–344 μg g⁻¹) reduced the dry weight (DW) production by 1.8–3.9 times and altered the plant development time (Table S1). These results clearly indicate that the non-excluder rice accessions suffered from Cd stress (Afzal et al., 2018; White, 2012a). Soil flooding increased the pH in the soil solution (Figure S2), decreased the Cd concentration in all rice shoots, and also attenuated the Cd stress in the non-excluder plants (Table 1). In the non-flooded soil, the soil pH temporarily dropped from 5.2 to 3.7 during rice growth and altered

Table 1
Rice dry weight, cadmium concentration, mass, and isotopes.

soil water management	rice accessions	plant part	dry weight		Cd concentration			Cd mass			Cd isotope ratio			
			[g]		[μg g ^{−1}]			[μg]			δ ^{114/110} Cd [‰]			
			mean	±sd ^a	mean	±sd ^a		mean	±sd ^a		mean	±sd ^a		
flooded	non-excluder	root	2.08	0.37	<i>b</i>	263	54.7	<i>c</i>	551	180	<i>c</i>	−0.42	0.02	<i>a</i>
		shoot	11.5	1.38	<i>b</i>	63.7	14.1	<i>b</i>	720	99.7	<i>b</i>	−0.33	0.02	<i>b</i>
		plant	13.6	1.28	<i>B</i>	94.8	24.4	<i>C</i>	1271	272	<i>B</i>	−0.37	0.01	<i>A</i>
	excluder	root	3.79	1.50	<i>a</i>	390	140	<i>c</i>	1329	175	<i>b</i>	−0.41	0.01	<i>a</i>
		shoot	20.7	1.59	<i>a</i>	8.57	0.70	<i>d</i>	178	18.9	<i>c</i>	−0.22	0.03	<i>a</i>
		plant	24.5	2.68	<i>A</i>	61.8	7.21	<i>D</i>	1507	171	<i>B</i>	−0.39	0.01	<i>B</i>
non-flooded	non-excluder	root	0.91	0.36	<i>c</i>	917	138	<i>a</i>	822	291	<i>c</i>	−0.55	0.03	<i>c</i>
		shoot	5.37	1.26	<i>c</i>	344	53.6	<i>a</i>	1884	668	<i>a</i>	−0.57	0.02	<i>c</i>
		plant	6.29	1.34	<i>C</i>	425	37.4	<i>A</i>	2705	793	<i>A</i>	−0.56	0.01	<i>D</i>
	excluder	root	4.55	0.70	<i>a</i>	706	74.6	<i>b</i>	3214	628	<i>a</i>	−0.47	0.01	<i>b</i>
		shoot	20.8	0.73	<i>a</i>	29.6	4.61	<i>c</i>	614	91.6	<i>b</i>	−0.31	0.03	<i>b</i>
		plant	25.3	0.66	<i>A</i>	151	24.9	<i>B</i>	3827	708	<i>A</i>	−0.45	0.01	<i>C</i>

^a sd = standard deviation of the mean of $n = 4$ experimental replicates. Letter indicate significant differences of the mean between plant parts (lowercase letters: italic = roots, regular = shoots) or between the entire plants (uppercase letters). Statistical differences of the mean were determined using Kruskal Wallis test (DW, concentration, Cd mass shoot, Cd mass plant) or ANOVA (Cd mass roots & Cd isotopes plant (transformation = \log_{10}), Cd isotopes roots ($1/x$), Cd isotopes shoots (no transformation)).

the Cd solubility (Figure S2). The pH shifts were likely induced by the N fertilization (details in SI.8).

3.2. Effect of water management on Cd isotopes in soil solution and rice

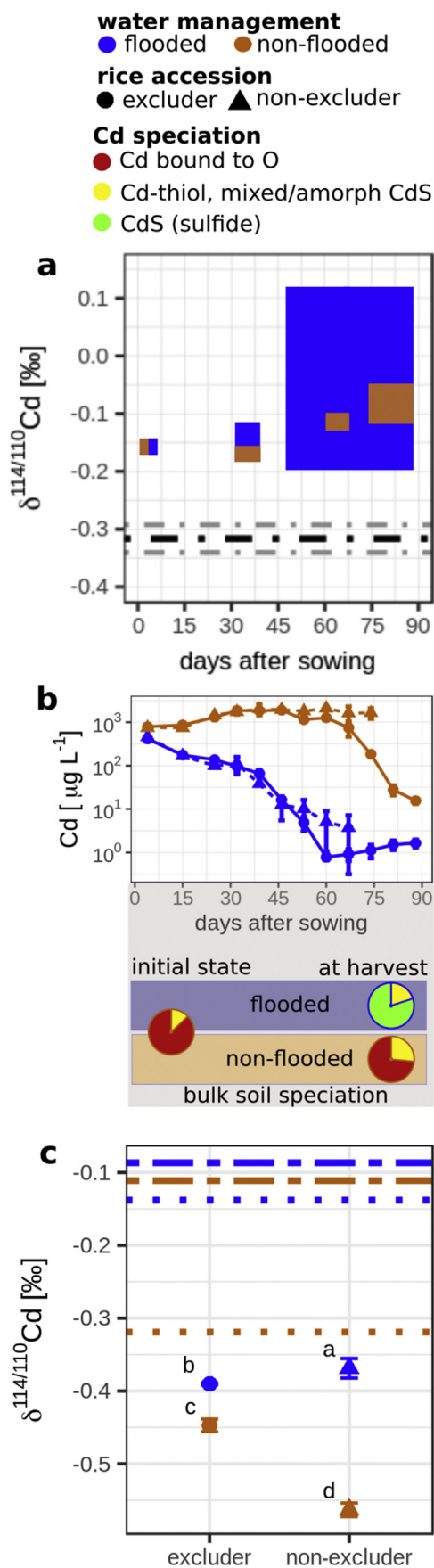
Irrespective of the water management, the soil solution was consistently enriched in heavy Cd isotopes when compared to the bulk soil ($\Delta^{114/110}\text{Cd}_{\text{soil.solution-bulk.soil}} = 0.17\text{--}0.21\text{‰}$, Fig. 1a). This enrichment was weaker than previously reported values for other soils ($\Delta^{114/110}\text{Cd}_{\text{solution/leachate-bulk.soil}} = 0.46\text{--}0.79\text{‰}$, Imseng et al., 2019; Zhang et al., 2016) and Cd sorption to Mn oxyhydroxide and precipitation in to Cd sulfides ($\Delta^{114/110}\text{Cd}_{\text{liquid-solid}} = 0.24\text{--}0.54\text{‰}$ Guinoiseau et al., 2018; Wasylenki et al., 2014). An explanation for the enrichment of heavy isotopes in soil solution could be that S donor groups of soil organic matter (SOM) can act as a major binding site for Cd in soils (Karlsson et al., 2007). Alternatively, Cd sorption to non-organic sorption sites such as birnessite (Mn-odxyhydroxide, Wasylenki et al., 2014), calcite (Horner et al., 2011), and sulfides (Guinoiseau et al., 2018) as well as its sorption to soils (Imseng et al., 2019; Wiggerhauser et al., 2016; Zhang et al., 2016) revealed an enrichment of heavy isotopes in the liquid phase. This enrichment was previously ascribed to the comparably short bond lengths of Cd and its oxygen donors in the hydrated Cd species (Imseng et al., 2019). In this study, Cd was bound to S with a Cd–S distance of 2.51 Å in the flooded soil and bound to O and S, with Cd–O and Cd–S bond lengths of 2.30 and 2.54 Å (Table S7, Imseng et al., 2019; Wasylenki et al., 2014). Reported values for aqueous Cd^{2+} are in the range of 2.27–2.28 Å (Bazarkina et al., 2010; Ohtaki and Johansson, 1981; Vasconcelos et al., 2008). Here, we found a longer bond length for aqueous Cd^{2+} (2.31 Å). We suspect that a thermal effect increased the Cd–O distance for this aqueous reference compound recorded at 10K (Figure S1d; Table S7b). In any case, our results revealed that the average bond lengths in both soils were longer than in aqueous Cd^{2+} and that this was mainly due to the presence of S ligands in the soils. Therefore, our results confirm that the enrichment of heavy isotopes in the soil solution compared to the bulk soil could be related to longer Cd bond lengths in the solid phase compared to the liquid phase of the soil.

Soil flooding started to change the chemical conditions in the soil after 46 DAS (Figure S2). From then on, the Eh strongly decreased while total Fe increased. Soil flooding further lead to a constant increase in pH in the soil solution (maximum pH = 6.4),

until the plants were harvested. The initial fraction of Cd in the soil solution compared to the total Cd in the bulk soil dropped from 5.82% to 0.84% (32–39 DAS) and then ended at 0.0012% (45–88 DAS, Fig. 1b, Table S9). In the same period, the Cd concentration in the soil solution strongly declined, and Cd in the solid phase of the soil was entirely bound to S (Fig. 1b). This indicates that Cd precipitation with sulfides occurred on the flooded soil and solution. In the same period, the mean isotope compositions in the soil solution increased slightly +0.12‰ but could not be distinguished from the isotope compositions measured before 45 DAS due to the large variation of Cd isotope compositions during 46–88 DAS ($\pm 0.15\text{‰}$, sd of $n = 2$ experimental replicates, Fig. 1a). The large error bar was likely caused by the merging of samples of different time points to one period (46–88 DAS). This merging procedure integrated the distinct temporal variations of Cd concentrations and isotope compositions of the experimental replicates (details in SI.2).

The Cd transfer from soil to rice was 2.1–2.5 times smaller in the flooded soil than in the non-flooded soil (Table 1). In addition, Cd isotope compositions $\delta^{114/110}\text{Cd}$ of rice were +0.06–0.19‰ heavier in the flooded soil compared to the non-flooded soil (Fig. 1c) while Cd speciation in the bulk soil changed from Cd–O (87%) to Cd–S (100%) during flooding (Fig. 1b). Particularly, Cd was associated to crystalline CdS (80%) and Cd thiols and mixed/amorphous CdS (Cd–S, 20%, Figure S1; Table S7). Previous studies have shown the formation of insoluble CdS in flooded soils (Fulda et al., 2013; Furuya et al., 2016). In addition, a batch study showed that the precipitation of Cd into CdS leads to a depletion of light isotopes in the liquid phase by $\Delta^{114/110}\text{Cd}_{\text{liquid-solid}} = 0.28\text{--}0.52\text{‰}$ (Guinoiseau et al., 2018). The different isotope compositions in the plants that grew under non-flooded and flooded conditions corresponded with the Cd sulfide batch study. During flooding, CdS precipitated in the flooded soils (Fig. 1b) and preferentially light isotopes were immobilized in the soil. This process likely promoted the transfer of heavy isotopes from soil to plant (Fig. 1c).

The difference of isotope compositions in the plants in this study ($\Delta^{114/110}\text{Cd}_{\text{plant.flooded-plant. non.flooded}} = 0.06\text{--}0.19\text{‰}$, Fig. 1c) was smaller than in the Cd sulfide batch study ($\Delta^{114/110}\text{Cd}_{\text{liquid-solid}} = 0.28\text{--}0.52\text{‰}$, Guinoiseau et al., 2018). This difference might be related to the fact that the rice plants took up Cd before the redox conditions in the soil significantly changed (Eh drop, pH increase, increasing Fe concentrations, Figure S2). Alternatively, soil flooding may have induced other processes that controlled the mobility and isotope fractionation of Cd between soil and plant such as an intermediate sorption of Cd to Fe and Mn oxides (J. Wang



et al., 2019). Sorption of Cd to Mn oxides yields, depending on the Cd speciation in the liquid phase, an apparent difference in isotope composition of $\Delta^{114/110}\text{Cd}_{\text{liquid-solid}} = 0.24\text{--}0.54\text{‰}$ (Wasylenki et al., 2014). More processes that potentially fractionated Cd isotopes before they were taken up by the rice plants are discussed in the next section.

3.3. Mechanisms that fractionate Cd isotopes on their pathway from soil to the rice root

Mechanisms other than changes in the Cd speciation in the solid phase of the soil may fractionate Cd isotopes from their pathway from the soil to the plant root (Imseng et al., 2019). Irrespective of the water management, the soil solution was enriched in heavy isotopes while plants were enriched in light isotopes compared to the bulk soil (Fig. 1a and c). Furthermore, the rice plants were strongly enriched in light isotopes compared to the soil solution ($\Delta^{114/110}\text{Cd}_{\text{rice-soil,solution}} = -0.31 \pm 0.08\text{‰}$, Fig. 1c). These results correspond with the results obtained for wheat and barley that grew in non- or less-contaminated soils ($\Delta^{114/110}\text{Cd}_{\text{plant-soil,solution}} -0.14 \pm 0.17\text{‰}$, Imseng et al., 2019) as well as hydroponically grown cacao ($\Delta^{114/110}\text{Cd}_{\text{plant-solution}} -0.22 \pm 0.08\text{‰}$), and Cd accumulator plants ($\Delta^{114/110}\text{Cd}_{\text{plant-solution}} -0.42 \pm 0.13\text{‰}$) that were exposed to high Cd concentrations (Moore et al., 2020; Wei et al., 2016, 2018). Among the treatments, $\Delta^{114/110}\text{Cd}_{\text{rice-soil,solution}}$ was larger in the non-flooded (-0.30 to -0.43‰) than in the flooded soil (-0.24 to -0.27‰ , Fig. 1c, Table S10), indicating that the water management also affects the Cd isotope fractionation between soil solution and plant. The putative mechanisms that controlled the isotope fractionation between soil and rice plants in addition to the Cd speciation in the solid phase are discussed in the following sections.

Additional Cd isotope fractionation steps can occur in the soil. The variation in Cd speciation in the liquid phase of the soil could fractionate Cd isotopes (Imseng et al., 2019). In the present study, hydrated Cd was the major Cd species in the soil solution regardless the treatment and sampling period (49–84%, details in Figure S3; Table S9). Therefore, binding of Cd to inorganic and organic ligands may have played a minor role for Cd isotope fractionation between the soil and the plant.

Additional Cd isotope fractionation steps can occur prior to Cd uptake by the root. In the root, Cd can be adsorbed to negatively charged surfaces in the apoplast. In aerated soils, pectins are a major component of root surfaces that contain carboxyl binding groups while the roots can become coated with re-precipitated oxyhydroxides in flooded soils (Khan et al., 2016; Sattelmacher, 2010). In the flooded soil of this study, the Fe concentration in the soil solution significantly increased (Figure S2) and Fe plaque was visible at harvest. We determined the Cd pool that is sorbed on the root surfaces by using a cation exchange approach (CaCl_2 extract, Straczek et al., 2008). At harvest, this Cd pool in the root was a minor fraction of the total quantity of Cd that accumulated in the root ($<1.55\%$, Table S11). This result is consistent with the root

Fig. 1. Cadmium in soil-rice systems. Rice was transplanted from the nursery to the soil at 25 days after sowing (DAS) and harvested when the rice was flowering. **a)** Cd isotope ratios in the soil solution at characteristic periods, areas represent \pm sd of the mean of $n \geq 2$ experimental replicates. The dashed lines represent the mean Cd isotope ratio of the bulk soil (\pm 2sd of $n = 2$ processing replicates). **b)** Cd concentration in the soil solution and Cd speciation in the bulk soil (results of the linear combination fits in Table S7). **c)** Cd isotope ratios in the whole rice plant that represents the Cd pool that was transferred from soil to rice. Error bars represent \pm standard deviation of $n = 4$ replicates. Letters indicate significant differences of the mean determined using Kruskal-Wallis test. Dashed lines represent the weighted mean isotope ratio in bulk soil solution (details in Fig. 1b). Dotted lines represent the isotope ratio in the CaCl_2 root extracts (details in Table S11).

XANES spectra that revealed that Cd was fully bound to S in the bulk roots and no Cd–O pool was detectable. Therefore, our data suggests that CaCl_2 extractable Cd from the roots represented a small and readily exchangeable Cd pool that was temporarily sorbed to root surfaces and available for plant uptake. In the non-flooded soil and in the flooded soil, root extracts were enriched in light isotopes when compared to the soil solution (Fig. 1c). In addition, this enrichment was larger in the non-flooded ($\Delta^{114/110}\text{Cd}_{\text{rootex-soil.solution}} -0.19$ to -0.22%) than in the flooded soils ($\Delta^{114/110}\text{Cd}_{\text{rootex-soil.solution}} -0.11$ to 0.01% , Table S11). This distinct light isotope enrichment could indicate that both pectins and reprecipitated oxyhydroxides preferentially adsorbed light isotopes from the solution, but to a larger extent for pectins. Alternatively, the distinct $\Delta^{114/110}\text{Cd}_{\text{rootex-soil.solution}}$ could have been induced by changes in the soil solution during soil flooding. When the root extracts were sampled, the soils were strongly reduced and most Cd was bound to S in the soil (Fig. 2b) and the fraction of Cd bound to DOM in the soil solution increased (Figure S3). These changes could have led to net enrichment of heavy isotopes in the hydrated Cd^{2+} species in the soil solution that is exchangeable with the cations that are sorbed to the root surface.

Uptake of Cd by rice is mainly controlled by OsNramp5 (Sasaki et al., 2012). In yeast, a mutant that contained a TcNramp5 transporter was $0.60\text{--}0.75\%$ ($\delta^{114/110}\text{Cd}$) lighter than its wild type (Moore et al., 2020). Thus, Cd absorbed by OsNramp5 likely contributed to the overall enrichment of light isotopes in rice.

3.4. Cadmium transport within rice

Cadmium mass balances revealed that the excluder rice accession with the functional OsHMA3 vacuolar transporter for Cd contained in average about 5.2–7.5 times more Cd in the roots than in the shoots (Fig. 2a). In contrast, there was consistently more Cd found in the shoots than in the roots of the non-excluder rice without the functional OsHMA3 vacuolar transporter. This finding corresponds with previous studies that identified the OsHMA3 tonoplast transporter as a controlling factor for Cd distribution between root and shoot (Shao et al., 2017; Ueno et al., 2010). In the roots, Cd was entirely bound to thiols regardless of the treatment (Fig. 2b). In this study, the mean apparent difference in isotope composition between the root and the shoot was significantly larger in the excluder rice ($\Delta^{114/110}\text{Cd}_{\text{shoot-root}} 0.16\text{--}0.19\%$) than in the non-excluder rice ($\Delta^{114/110}\text{Cd}_{\text{shoot-root}} -0.02$ to 0.08% , Fig. 2b, Table S12). A Rayleigh model accurately described that the isotope fractionation was induced by the progressive retention of Cd during root radial transfer (Fig. 2c, details in SI.7). The model predicts an isotope fractionation of -0.08% for Cd retention in the roots in relation to the fraction of Cd in the plant that is retained in roots (Aucour et al., 2011).

The retention of light Cd isotopes in the roots of the excluder rice coincides with the Cd isotope fractionation in wheat and barley that grew on soils with background Cd concentrations ($>0.5\text{ mg kg}^{-1}$) or less Cd contaminated soils ($<1.5\text{ mg kg}^{-1}$, Imseng et al., 2019; Wiggerhauser et al., 2016) and cacao seedlings that were cultivated in hydroponics ($2.2\text{ }\mu\text{g L}^{-1}$ Cd, Moore et al., 2020). The similar isotope fractionation pattern in these plants and in the excluder rice implies that similar processes retain Cd in the roots regardless of the Cd concentration in the soil solution or hydroponic nutrient solution. In this study, the apparent difference in isotope composition $\Delta^{114/110}\text{Cd}_{\text{shoot-root}}$ of the non-excluder rice ($0.16\text{--}0.19\%$) was significantly larger than that of the non-excluder rice (-0.02 to 0.08% , Table S12) without a functional OsHMA3 that is localized at the root tonoplast (Ueno et al., 2010). These results suggest that the processes that were involved into vacuolar sequestration of Cd induced the Cd isotope fractionation between

root and shoot. However, we cannot exclude that Cd stress altered the $\Delta^{114/110}\text{Cd}_{\text{shoot-root}}$ in the non-excluder plants (Table 1). In non-excluder rice, the $\Delta^{114/110}\text{Cd}_{\text{shoot-root}}$ was larger in the flooded soils ($+0.08 \pm 0.04\%$) than in the non-flooded soils ($-0.02 \pm 0.05\%$) while the Cd concentration in the roots was 2.5 times smaller in the rice that grew on the flooded soil compared to the non-flooded soil. Nevertheless, the distribution of Cd between root and shoot (Fig. 2a) and the Cd speciation in the roots were identical among the non-excluder rice (Fig. 2b) while the distribution of Cd in the both rice accessions and its corresponding isotope fractionation was very systematic (Fig. 2c). The putative mechanisms that are involved into the retention of light isotopes in the rice roots are discussed in the following sections and summarized in Fig. 3.

The transport and storage of Cd into the root vacuole requires a membrane transport that is mainly facilitated by OsHMA3 (Shao et al., 2017). Similar to OsNramp5, the membrane protein OsHMA3 may favor light isotopes for membrane transport (Moore et al., 2020). Thus, the vacuolar transport of Cd by OsHMA3 could explain the enrichment of light isotopes in the root of the excluder rice. However, the two transporters and their chemical environments differ from each other. OsNramp5 is a transporter that transports Cd from the soil solution into the cytosol towards an electrochemical gradient while OsHMA3 can pump Cd from the cytosol into the vacuole against such a gradient (Olsen and Palmgren, 2014; Østerberg and Palmgren, 2018). Furthermore, the donor atoms and coordination of the metal binding sites may differ (Ehrnstorfer et al., 2014; Leitenmaier et al., 2011; Wang et al., 2014). Finally, Cd was mostly present as hydrated Cd in the soil solution while Cd in the root cytosol and vacuole was fully bound to S (Fig. 2b, S3). The isotope fractionation during transport into the vacuole is therefore the result of Cd dissociation from Cd–S in the cytosol, binding to the strong metal binding sites of OsHMA3, followed by a transport into the vacuole (Wang et al., 2014; Zhao et al., 2016). Since this chain of processes is fast and rather kinetically than thermodynamically controlled, transport into the vacuole may favor light isotopes (Köbberich and Vance, 2017). However, the dissociation of Cd from S could diminish the enrichment of light isotopes during membrane transport since Cd–S complexes with heavy isotopes are less stable than complexes with light isotopes (Yang et al., 2015; Zhao et al. in revision). Finally, a similar type of transporter is involved in Cd transport from root to shoot (OsHMA2, Nocito et al., 2011; Satoh-Nagasawa et al., 2012), which could partly outbalance the isotope fractionation that is induced by OsHMA3.

Metal chelators that form stable Cd–S complexes in roots could also be a main factor that immobilizes Cd in roots (Cao et al., 2018; Nocito et al., 2011). Theoretical calculations suggest that Cd chelating thiol complexes such as phytochelatin are strongly enriched in light isotopes when compared to non-chelating thiols such as cysteine and glutathione as well as Cd–O complexes (including hydrated Cd^{2+} , Yang et al., 2015; Zhao et al. in revision). Thus, the enrichment in light isotopes in the roots with functional OsHMA3 could be due to Cd binding to chelators such as phytochelatin in the vacuole (Cao et al., 2018; Horner et al., 2013; Nocito et al., 2011). These chelators are generally more stable than other thiol complexes in a wide range of pH (Dorčák and Krěžel, 2003). Hence, heavier Cd that is bound to weaker thiol complexes such as cysteine or glutathione can be transported from the vacuole back into the cytosol (Fig. 3).

Our Cd speciation and isotope results suggest that Cd–S complexes can be divided into stable and less stable forms. Less stable forms may buffer Cd toxicity especially in the cytosol while stable forms sequester Cd (Clemens, 2019; Nocito et al., 2011). This suggestion is supported by Yan et al. (2016). In their study, Cd was entirely bound to S in the excluder rice, however, a minority of Cd

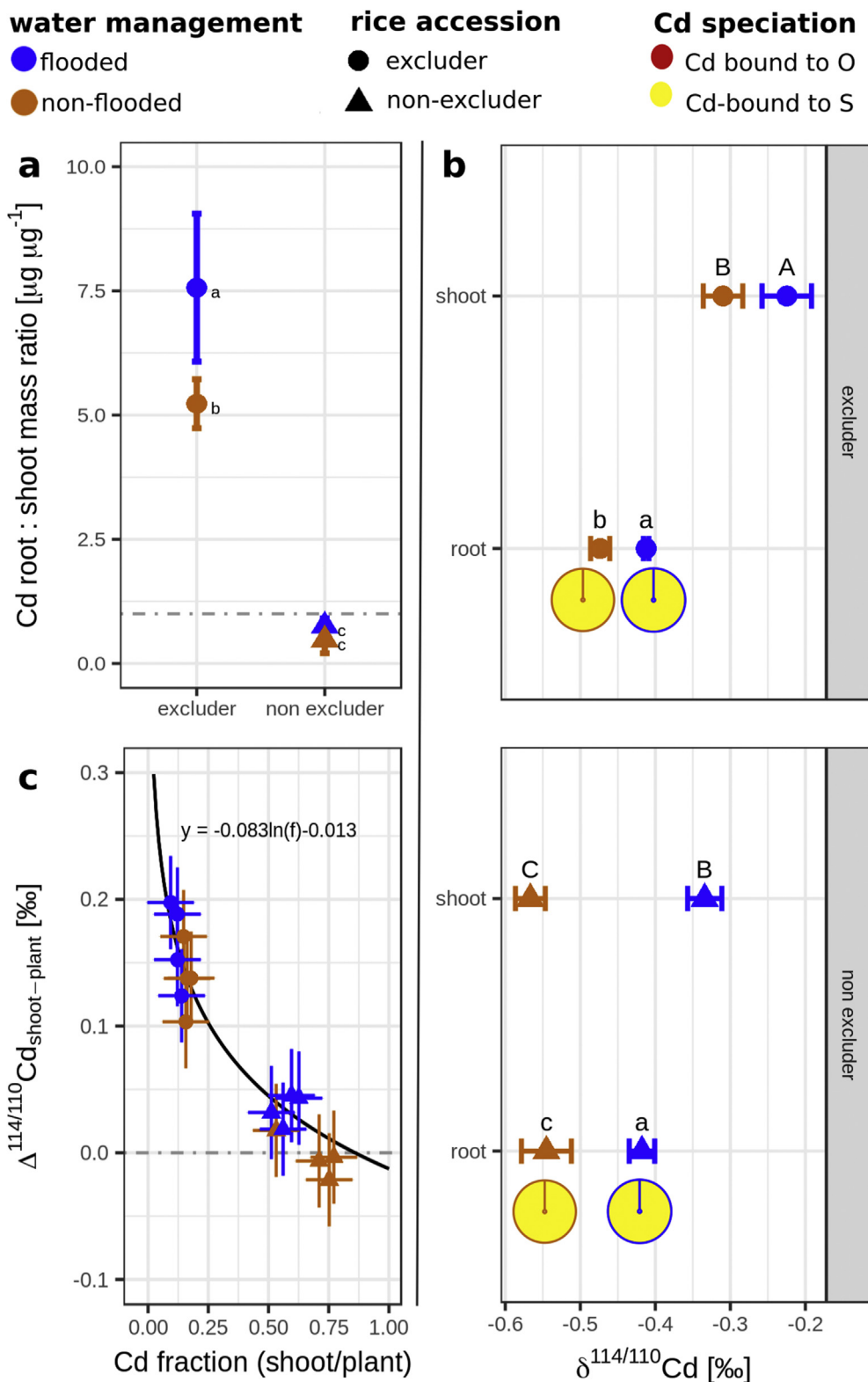


Fig. 2. Cadmium in rice accessions with and without functional vacuolar OsHMA3 membrane transporters grown on soils with distinct water management. Excluder = rice with functional OsHMA3, non-excluder = rice without functional OsHMA3. **a)** Cd mass ratio between root and shoot, error bars denote standard deviation of $n = 4$ experimental replicates, letters denote statistical difference of the mean determined with ANOVA (log10 transformed). **b)** Cd isotope ratios in rice, pie charts show Cd speciation (grouped into Cd bound to sulfur (S) and Cd bound to oxygen (O)). Error bars represent standard deviation of $n = 4$ experimental replicates, letters denote statistical difference of the mean determined with ANOVA for either roots (lowercase letters, 1/x transformed) or shoots (uppercase letters, non transformed). **c)** Relation of Cd isotope fractionation and Cd distribution with non-linear rayleigh fit that describes isotope fractionation factor ϵ (details SI.7). Error bars represent propagated 2sd for $n = 1$ experimental replicate based on the experimental precision of this study (details eq. S1).

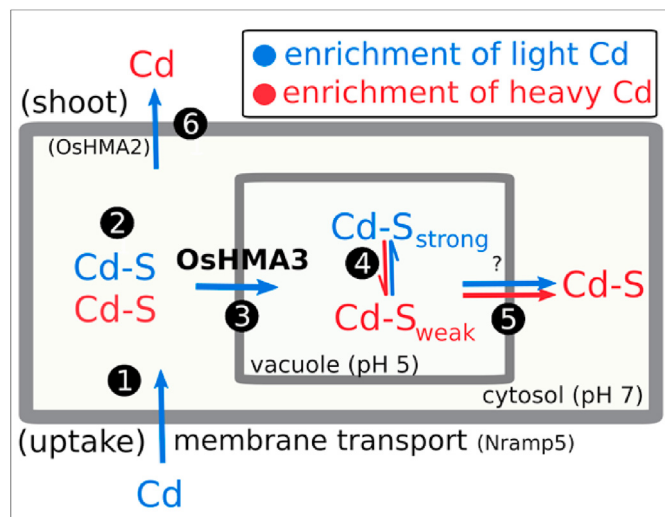


Fig. 3. Preliminary conceptual model of Cd isotope fractionation in a rice root with functional OsHMA3 (excluder). The model shows a simplified rice root cell, Cd-S = Cd bound to a sulfur donor atoms. 1) Preferentially light Cd isotopes are taken up into the cytosol with micro nutrients transporter (OsNramp5, Moore et al., 2020; Sasaki et al., 2012). 2) Cd is bound to S donors in the root cytosol, likely to prevent Cd binding to e.g. proteins (Clemens, 2019). 3) Preferentially light isotopes accumulate in the vacuoles. If OsHMA3 is functioning, most Cd may be immobilized in the vacuoles. Transport by OsHMA3 involves binding to the metal binding site of the transporter which requires a ligand exchange of Cd in the cytosol (Cd-S) prior to membrane transport (Wang et al., 2014; Zhao et al., 2016). The entire process is partly kinetically controlled and may favor light isotopes (Köbberich and Vance, 2017). 4) Stable ligands with S donor atoms favor light isotopes which leads to an enrichment of heavy Cd in complexes with less stable S donor ligands (Zhao et al. in revision). 3-4) If OsHMA3 is not functional (non-excluder), significantly less Cd is transported into the vacuole which decreases the apparent difference in isotope compositions between root and shoot. 5) Cd in less stable complexes is more likely to be transported by membrane proteins than stable complexes (Clemens, 2019; Yan et al., 2016). The Cd bound to weaker S donors that is enriched in heavy isotopes can be remobilized from the vacuole into the cytosol by a yet unknown transporter (W. Wang et al., 2019). This leads to the enrichment of heavy isotopes into the cytosol 6) that can be further transported from root to shoot by OsHMA2 that requires a ligand exchange (see point 3 for OsHMA3, Nocito et al., 2011; Satoh-Nagasawa et al., 2012).

(10–15%) was bound to O in the non-excluder rice. Cd–O was ascribed to Cd that was bound to less stable complexes in the cytosol and that can be transported from root to shoot. In our study, Cd was entirely bound to S in roots, which suggests that Cd was bound to S in the cytosol. This was probably due to intense S nutrition in our soil-rice systems (Table S8).

3.5. Environmental implications

We combined isotope analysis with solid state speciation analysis to advance Cd isotope process tracing in soil-plant systems. Our results confirmed that S can play a key role in immobilizing Cd in soils (Fulda et al., 2013; Karlsson et al., 2005) and in rice (Cao et al., 2018; Nocito et al., 2011) in systems that are rich in Cd and S. During soil flooding, Cd precipitated as CdS and the rice became enriched in heavy isotopes compared to the soil that was not flooded. We confirmed that plants take up light isotopes (Imseng et al., 2019; Moore et al., 2020; Wei et al., 2016; Wiggerhauser et al., 2016) regardless the water management. We further highlighted that this enrichment is, in addition to membrane transport (Moore et al., 2020), also induced by the sorption of Cd to root surfaces. Within the rice, light isotopes were retained in the roots, as it was previously shown for other cereals and cacao seedlings (Imseng et al., 2019; Moore et al., 2020; Wiggerhauser et al., 2016). Based on a comparison of rice plants with and without functional vacuolar Cd

transporter OsHMA3 and Cd speciation analysis, we proposed that membrane transport and Cd chelation by thiols retain Cd and its light isotopes in root vacuoles.

Our study illustrated how Cd isotopes can provide complementary information to Cd speciation and Cd mass balances, thereby identifying processes that control the mobility of Cd in soil-plant systems. This study partly confirmed the view that Cd binding to S can be a major driver of Cd isotope fractionation. Our results also illustrated that additional processes to Cd–S binding occur in parallel that potentially attenuated or amplified the mobility of Cd and the extent of Cd isotope fractionation. For instance, soil flooding not only changed the Cd speciation in the solid soil but also $\Delta^{114/110}\text{Cd}_{\text{rice-soil solution}}$. In addition, vacuolar transport may include the dissociation of heavy Cd isotopes from a S donor atoms prior to membrane transport and storage in the vacuole. More studies on simpler systems need to be conducted to further differentiate processes that fractionate Cd in complex soil-plant systems. This includes batch (Guinoiseau et al., 2018; Marković et al., 2017; Wasylenki et al., 2014) and theoretical studies (Yang et al., 2015) that extend the database of Cd isotope fractionation factors of Cd sorption to minerals and binding to organic ligands. Additionally, there is a need for studies that gradually approach the complexity of soil-plant systems such as Cd sorption to soil composites (Du et al., 2016), artificial membranes that mimic plant uptake (Vera et al., 2018) or yeast mutant studies (Moore et al., 2020). These types of approaches will complement studies on soil-plant systems and further advance the use of Cd isotopes and the understanding of the processes that control the mobility of Cd in the environment.

Author Statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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