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Cadmium uptake and transport processes in rice revealed by stable isotope fractionation and Cd-related gene expression

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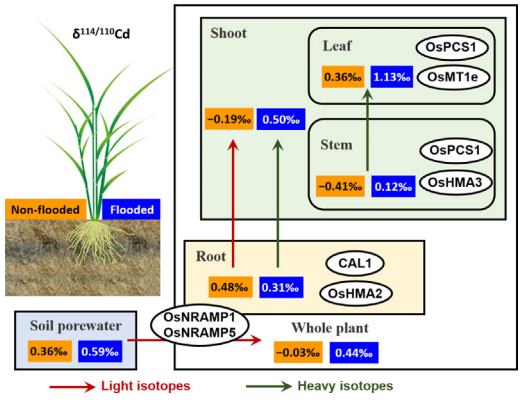
HIGHLIGHTS

- Up-regulation of *OsNRAMP1* and *OsNRAMP5* in roots facilitated light Cd isotope uptake.
- Up-regulation of *CAL1* in roots favored transfer of light Cd isotope to shoots.
- Stems retained the majority of Cd and preferred lighter Cd isotopes relative to leaves.
- Light Cd isotopes in older leaves may ascribe to up-regulation of *OsMIT1e*.
- Isotopically light Cd like Cd-PCs could be transported from older leaves to younger leaves.

GRAPHICAL ABSTRACT



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ABSTRACT

Multiple processes are involved in Cd transfer in rice plants, including root uptake, xylem loading, and immobilization. These processes can be mediated by membrane transporters and can alter Cd speciation by binding Cd to different organic ligands. However, it remains unclear which processes control Cd transport in rice in response to different watering conditions in soil. Herein, Cd isotope fractionation and Cd-related gene expression were employed to investigate the key regulatory mechanisms during uptake, root-to-shoot, and stem-to-leaf transport of Cd in rice grown in pot experiments with Cd-contaminated soil under flooded and non-flooded conditions, respectively. The results showed that soil flooding decreased the Cd concentration in soil porewater and, thereby, Cd uptake and transport in rice. Cd isotopes fractionated negatively from soil porewater to the whole rice (flooded: $\Delta^{114/110}Cd_{rice\text{-}porewater} = -0.15‰$, non-flooded: $\Delta^{114/110}Cd_{rice\text{-}porewater} = -0.39‰$), suggesting that Cd transporters preferentially absorbed light Cd isotopes. The non-flooded treatment revealed an upregulated expression of *OsNRAMP1* and *OsNRAMP5* genes compared to the flooded treatment, which may partially contribute to its more pronounced porewater-to-rice fractionation. Cd isotopes fractionated positively from roots to shoots under flooded conditions ($\Delta^{114/110}Cd_{shoot\text{-}root} = 0.19‰$). However, a reverse direction of fractionation was observed under non-flooded conditions ($\Delta^{114/110}Cd_{shoot\text{-}root} = -0.67‰$), which was associated with the substantial upregulation of *CAL1* in roots, facilitating xylem loading of Cd-CAL1 complexes with lighter isotopes.

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After being transported to the shoots, the majority of Cd were detained in stems (44%–55%), which were strongly enriched in lighter isotopes than in the leaves ($\Delta^{114/110}Cd_{leaf\text{-}stem}$ = 0.77 to 1.01‰). Besides the Cd-CAL1 transported from the roots, the expression of *OsPCS1* and *OsHMA3* in the stems could also favor the enrichment of Cd-PCs with lighter isotopes, leaving heavier isotopes to be transported to the leaves. The higher expression levels of *OsMT1e* in older leaves than in younger leaves implied that Cd immobilization via binding to metallothioneins like OsMT1e may favor the enrichment of lighter isotopes in older leaves. The non-flooded treatment showed lighter Cd isotopes in younger leaves than the flooded treatment, suggesting that more Cd-CAL1 in the stems and Cd-PCs in the older leaves might be transported to the younger leaves under non-flooded conditions. Our results demonstrate that isotopically light Cd can be preferentially transported from roots to shoots when more Cd is absorbed by rice under non-flooded conditions, and isotope fractionation signature together with gene expression quantification has the potential to provide a better understanding of the key processes regulating Cd transfer in rice.

1. Introduction

Cadmium (Cd) is a trace element prone to accumulation in plants, especially in Cd-contaminated areas, which poses a threat to human health via the food chain (Meharg et al., 2013; Zhao et al., 2015). Rice is a significant dietary source of Cd, particularly in Asian populations, and long-term consumption of Cd-contaminated rice grains can cause severe health problems such as chronic damage to the kidneys and bones (Hu et al., 2016). It is of great importance to understand the crucial processes that regulate Cd transport in rice to alleviate Cd accumulation in rice grains.

Recently, Cd stable isotope signatures have been used to trace the transfer processes of Cd in plants (Imseng et al., 2019; Wei et al., 2018; Wiggenhauser et al., 2016), which can provide insights into the cellular and molecular processes involved in the uptake and sequestration of Cd in rice (Wiggenhauser et al., 2021a, 2021b; Zhang et al., 2021). In general, Cd isotope compositions in whole plants are lighter than those in soil porewater or nutrient solution, which is associated with a number of processes in the soil and root uptake by membrane transporters (Imseng et al., 2019; Wei et al., 2018). Although soils preferentially release isotopically heavy Cd into the soil porewater, light isotopes diffuse rapidly towards and are preferentially adsorbed on the root surface (Imseng et al., 2019). When compared to flooding practice, the soil pH decreases and the concentration and bioavailability of Cd^{2+} increase under drainage practice because of the oxidation of sulfides and Cd desorption from Fe—Mn minerals (Fulda et al., 2013; Wang et al., 2019). The membrane transporters that can mediate Cd uptake and transport in rice are primarily responsible for the homeostasis of micronutrients, such as iron transporter OsNRAMP1, manganese transporter OsNRAMP5, and zinc transporters OsHMA3 and OsHMA2 (Cai et al., 2019; Sasaki et al., 2012; Takahashi et al., 2011, 2012). The NRAMP5 transporter in cacao plants has been reported to facilitate uptake of isotopically light Cd (Moore et al., 2020). When compared with wild-type rice, a rice accession without a functional OsHMA3 transporter for vacuolar sequestration showed smaller fractionation of Cd isotopes between roots and shoots, whereas overexpression of the *OsHMA3* gene caused an enrichment of heavier Cd in the leaves (Wiggenhauser et al., 2021a; Zhang et al., 2021). Cd is mainly bound to thiol-containing peptides such as phytochelatins (PCs) in the form of Cd—S in rice roots (Clemens and Ma, 2016; Wiggenhauser et al., 2021a). Lighter Cd isotopes preferentially bind to sulfur donor atoms of organic ligands, which show longer bond lengths and lower reduced partition function ratios according to calculations using density functional theory (Zhao et al., 2021). Therefore, the retention of light Cd isotopes in the roots has been suggested to be due to the membrane transport of Cd^{2+} by the OsHMA3 transporter and/or chelating Cd—S complexes in the vacuoles.

Rice plants can develop various strategies to change the chemical speciation and location of Cd in order to alleviate the toxicity of Cd, once Cd^{2+} is inadvertently absorbed into root cells via transporters like OsNRAMP1 and OsNRAMP5 (Chang et al., 2020; Sasaki et al., 2012; Takahashi et al., 2011). First, one of the detoxification pathways is the complexation of Cd with thiol compounds such as phytochelatins (PCs) and glutathione (GSH; a substrate for PC synthesis), which are enzymatically synthesized by the OsGS and OsPCS proteins, respectively (Park et al., 2017; Yamazaki et al., 2018). The expression levels of both *OsGS* and *OsPCS* genes in roots and shoots can be stimulated by Cd stress, and GSH biosynthesis appears to be more important in tolerance to Cd in rice, not only as a substrate for PC synthesis, but also as a ligand binding to Cd (Yamazaki et al., 2018). Second, Cd binding to metallothionein proteins is another important way to maintain intracellular metal homeostasis and eliminate heavy metal toxicity (Ogo et al., 2014; Rono et al., 2021). Some metallothionein-like genes such as *OsMT1e*, *OsMTI-1b*, and *OsMT-3a* have been identified in rice, and their expression in roots and shoots can be induced by Cd exposure (Malekzadeh et al., 2020; Mekawy et al., 2018; Rono et al., 2021). Although xylem loading of Cd^{2+} via OsHMA2 facilitates long-distance transfer of Cd to the leaf cells, the formation of Cd-binding MTs in the cytoplasm and/or nucleus could restrict the mobility of Cd in leaves (Mekawy et al., 2018; Rono et al., 2021). Third, the CAL1 protein in the cytosol of root parenchyma cells and leaf sheath can bind Cd^{2+}, forming Cd-CAL1 complexes (Luo et al., 2018). These complexes can be secreted into the xylem, followed by transport within the transpiration stream to the leaves. Therefore, CAL1 can facilitate the root-to-shoot transfer of Cd. Within the shoots, the Cd-CAL1 complexes are supposed to accumulate in the apoplast and prevent Cd loading into the phloem for Cd accumulation in rice grains (Luo et al., 2018; Zhao and Huang, 2018). Therefore, organic ligands such as PCs, metallothioneins, and CAL1 play crucial roles in the transport and storage of Cd in rice plants.

Interpretation of Cd isotope signatures is generally challenging in complicated soil-rice systems because multiple processes are involved in Cd transport within rice, which are mediated by a number of membrane transporters and organic ligands (Clemens and Ma, 2016; Imseng et al., 2019; Wiggenhauser et al., 2021a; Zhang et al., 2021). We hypothesize that marked changes in gene expression levels of the transporters and ligands may strongly impact the Cd distribution and isotope fractionation within rice, which potentially overcomes the limits of isotope process tracing in plants. In this study, Cd concentration measurements, isotope fractionation analyses, and gene expression quantification were performed for a rice variety grown in a pot experiment with Cd-contaminated paddy soil under flooded and non-flooded conditions, respectively. The non-flooded treatment was expected to show a higher uptake of Cd by rice than the flooded treatment. Results obtained from roots, stems, and individual leaves will provide detailed evidence to elucidate the dominant processes during Cd uptake, root-to-shoot transport, and distribution between stems and leaves in rice.

2. Materials and methods

2.1. Rice growth experiments

The paddy soil was obtained from the plow layer (0–20 cm) of a Cd-contaminated field for rice cropping in Shaoguan City (24.635485°N,

113.567534°E). The soil pH, concentrations of DTPA extractable Cd and total Cd were 6.15, 0.61 mg kg^{-1} and 1.04 mg kg^{-1}, respectively. The soil was air-dried, homogenized, and sieved to <5 mm before the pot experiments. Seedlings of rice (*O. sativa*) cultivar Huanghuazhan were germinated at 25 °C in a biochemical incubator and transferred to seedling-raising plates filled with uncontaminated soil according to previously reported protocols (Zhang et al., 2019). Thereafter, the rice seedlings were transplanted into pots filled with 12 kg of paddy soil in a greenhouse. The temperature and relative humidity during rice growth were within the range of 19–35 °C and 74.2%–79.8%, respectively. Flooded and non-flooded treatments were performed, in which deionized water (> 18.2 MΩ) was added to achieve a water level of approximately 5 cm above the soil surface and maintained 75% soil moisture content on a daily basis. Each watering treatment contained three pots with three seedlings in each pot, which were randomly placed. To ensure regular rice growth, urea, KH_2PO_4 and $K_2HPO_4 \cdot 3H_2O$ were added at a rate of 0.22 g kg^{-1}, 0.038 g kg^{-1} and 0.34 g kg^{-1} soil (dry weight), respectively.

2.2. Sample preparation and cadmium measurement

Rhizon samplers that were made of a male luer lock, a 10 cm porous part, and 12 cm PVC tubing (Rhizosphere Research Products, Netherlands) were inserted into the soil rhizosphere for soil porewater collection. Rice was harvested after 50 days of incubation when an elongation of the stem between the fourth and fifth leaves was observed, referred to as the jointing stage. The rice plants were divided into roots, stems, and leaves using ceramic scissors. A total of five leaves were collected from each plant, which were sequentially named the first, second, third, fourth, and fifth leaves from top to bottom, respectively (Fig. S1). Cd adsorbed on the root surface was extracted using 0.5 M HCl and 0.1 M HCl in sequence with ultrasonic cleaning for 15 min and twice for each treatment (Garnier et al., 2017).

Three experimental replicates from individual pots were collected and divided into three parts: one for Cd concentration determination, one for Cd isotope measurement, and the other for gene quantification. The average values and standard deviations of the Cd concentration and gene expression were determined by measurements of the three experimental replicates, while samples from the three experimental replicates were combined and analyzed in triplicate to measure the Cd isotopic composition (Liu et al., 2020a; Lv et al., 2021). Rice plant samples (1.0 g) were digested using a high-performance microwave digestion system (Milestone, ETHOS UP, Italy) according to previously reported protocols (Liu et al., 2020b). The concentrations of Cd in porewater, root HCl extract, and rice samples from three individual replicates of pots were determined by inductively coupled plasma mass spectrometry (NexION 300×, PerkinElmer, USA).

2.3. Chemical purification and cadmium isotope analysis

All the digested samples that contained more than 100 ng Cd were equilibrated with ^{111}Cd—^{113}Cd double-spike solution overnight and anion exchange resin AG1-X8 (100–200 meshes, Bio-Rad®, USA) were used for Cd separation from matrix elements according to previously reported protocols (Liu et al., 2020a). This method requires that the Cd concentrations of the sample solutions are higher than 20 ng g^{-1}, and has been successfully applied in the measurement of Cd isotopes in soil and plant samples recently (Lv et al., 2021; Zhou et al., 2020). Briefly, the resin was sequentially rinsed with ultrapure water, 1 N HNO_3, and 6 N HCl. Then, 6 N HCl and 0.3 N HCl were successively added to elute matrix elements including Na, Mg, Ca, Al, Ti, Zr, Fe, Ga, Ag, Pd, Mo, and In. Thereafter, a mixed acid containing 0.5 N HNO_3 and 0.1 N HBr was conducted to remove Zn and Sn, and 10 mL of 2 N HNO_3 was applied to collect purified Cd. After drying, the sample was re-dissolved in 2% m/m HNO_3 for Cd isotope analysis using a multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS, Neptune Plus, Thermo Fisher Scientific, USA) at the University of Science and Technology of China, Hefei, China. The Cd recoveries of plant reference materials were 96.0%–97.2% for citrus leaf (GBW10020, 0.17 ± 0.02 mg Cd/kg) and laver (GBW10023, 0.57 ± 0.05 mg Cd/kg), which verified the procedure of sample digestion and chemical purification.

During Cd isotope analysis, slightly modified procedures were carried out based on a previous study by Liu et al. (2020a). Measurements were performed in a low-resolution mode with Ni H-skimmer cones (Thermo Fisher Scientific) with high sensitivity and a stable signal to ensure the sensitivity of ^{114}Cd was ~120 V/ppb. The samples and standards were diluted to 50–100 ng g^{-1} before measurement. The pure Cd solution (BAM I012 Cd, Münster Cd, or AAS Cd) was analyzed for every four samples. To avoid cross-contamination between samples, the introduction system was cleaned using 5% HNO_3 *m/m* and 2% HNO_3 *m/m* in sequence for approximately 60 s until the ^{114}Cd signal was <3 mV. The data for each sample were obtained after 30 cycles with an integration time of 4.194 s for each cycle.

Procedural blanks induced by acid digestion and matrix separation were monitored before isotopic analyses ($n = 5$), whose total procedural Cd ranged from 0.10 ng to 0.20 ng and had a negligible impact on the Cd isotopic ratio (< 0.2‰ of the Cd mass in each sample). The precision and accuracy of the Cd isotope measurements were performed using replicate runs of an in-house standard (BAM I012 Cd and AAS Cd) and Münster Cd reference solutions, which revealed that the measurement precision was within ±0.05‰ (2sd). Duplicates from the BAM I012 Cd, AAS Cd and Münster Cd reference solutions yielded values of −1.3 ± 0.05‰ ($n = 154$), −0.69 ± 0.06‰ ($n = 170$) and 4.5 ± 0.05‰ ($n = 113$), respectively, which were in good agreement with previous results (Abouchami et al., 2013; Liu et al., 2020a; Zhou et al., 2020). Repetitive analyses of soil materials, i.e., NIST 2710, NIST 2710a, NIST 2711 and NIST 2711a with Cd concentrations of 12.3–54.1 mg kg^{-1} yielded average $\delta^{114/110}Cd$ values of −0.18 ± 0.00‰, −0.13 ± 0.05‰, 0.65 ± 0.01‰ and 0.54 ± 0.07‰ relative to NIST SRM 3108, respectively. These results are in good agreement with previous studies that showed $\delta^{114/110}Cd$ values of −0.18 ± 0.06‰, −0.20 ± 0.05‰, 0.63 ± 0.04‰ and 0.55 ± 0.05‰, respectively (Liu et al., 2020a; Zhou et al., 2020). Samples from three individual pots were combined and analyzed in triplicate. Analytical replicates from the standard materials and samples yielded an analytical reproducibility of 2sd < 0.08‰. The $\delta^{114/110}Cd$ values of an experimental replicate of root HCl extract, root and the third leaf under flooded conditions, and of root HCl extract under non-flooded conditions were − 0.11 ± 0.03‰, 0.29 ± 0.02‰, 1.72 ± 0.03‰ and 0.02 ± 0.06‰, respectively. These results verify the reliability of the Cd isotope compositions listed in Table S1.

2.4. Calculations

The Cd isotopic compositions are expressed as $^{114}Cd/^{110}Cd$ relative to the reference material NIST SRM-3108 as the Cd standard in ‰ using the following equation:

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

The apparent isotope fractionation from compartments A to B, including soil and plant compartments in the soil-rice system, was calculated using the Δ notation:

$$\Delta^{114/110}Cd_{A-B} = \delta^{114/110}Cd_A - \delta^{114/110}Cd_B \quad (2)$$

The $\delta^{114/110}Cd$ values for the whole rice and different parts of the rice plant were determined using weighted mean calculations with the Cd mass as a weighting factor as follows:

$$\delta^{114/110}Cd_{\text{whole rice (leaves,shoot,or grain)}} = \frac{\sum_i m_i c_i \delta^{114/110} Cd_i}{\sum_i m_i c_i} \quad (3)$$

where m represents the dry weight (g), c represents the Cd concentration (mg kg^{-1}), and i represents the different plant parts of the whole rice (root + stem + total leaf), of the shoot (stem + total leaf), or of the total leaves (first leaf + second leaf + third leaf + fourth leaf + fifth leaf).

The standard errors for Cd isotope data were calculated following the combination of error propagation of addition, multiplication, and division as follows:

$$SD_{i+j} = \sqrt{(SD_i)^2 + (SD_j)^2} \quad (4)$$

$$SD_{i\times j} = Mean_i \times Mean_j \times \sqrt{\left(\frac{SD_i}{Mean_i}\right)^2 + \left(\frac{SD_j}{Mean_j}\right)^2} \quad (5)$$

$$SD_{i/j} = \frac{Mean_i}{Mean_j} \times \sqrt{\left(\frac{SD_i}{Mean_i}\right)^2 + \left(\frac{SD_j}{Mean_j}\right)^2} \quad (6)$$

2.5. Gene quantification

The root, stem, and individual leaves were collected and frozen in liquid nitrogen for RNA extraction following the manufacturer's protocols (Omega Bio-Tek, Norcross, USA). The RNA samples were converted into cDNA using a reverse transcriptase kit (Takara, Kyoto, Japan), followed by qRT-PCR analysis using iCycler iQ Multi Color Real Time PCR (Bio-Rad, Hercules, CA, USA). The $2^{-\Delta\Delta Ct}$ method was used to evaluate the relative expression levels of genes normalized to the internal control gene *OsActin1*, and the expression of *OsActin1* was unaffected by the experimental treatment (Livak and Schmittgen, 2001). The average values and standard errors were estimated based on three independent biological replicates. Sequences of the primer pairs used in this study are listed in Table S2.

$$\Delta C_t = C_{(t,\text{target gene})} - C_{(t,\text{internal control gene})} \quad (7)$$

$$\Delta\Delta C_t = \Delta C_{(t,\text{Non-flooded})} - \Delta C_{(t,\text{Flooded})} \quad (8)$$

$$\text{Relative expression level} = 2^{-\Delta\Delta Ct} \quad (9)$$

$C_{(t,\text{ target gene})}$ and $C_{(t,\text{ internal control gene})}$ are the threshold cycles of the target gene and actin amplification, respectively. $\Delta C_{(t,\text{ Non-flooded})}$ and $\Delta C_{(t,\text{ Flooded})}$ are equal to the difference in threshold cycles for the target and internal control genes in the non-flooded and flooded treatments, respectively.

2.6. Statistical analysis

The normal distribution and homogeneity of variance were determined by the normality and Levene tests, respectively, using SPSS version 22.0. Then, an independent-sample *t*-test was used to analyze the significant differences in Cd concentrations in porewater, root HCl extract, and rice compartments, dry weight and Cd mass between flooded and non-flooded treatments, and the relative expression levels of genes among various rice compartments ($P < 0.05$).

3. Results

3.1. Cadmium concentration and mass

Cd concentration in soil porewater slightly increased over time under non-flooded conditions, while it slightly decreased under flooded conditions (Fig. S2). At harvest (50 d), the Cd concentration in soil porewater was 0.80 ± 0.04 μg L^{-1} under non-flooded conditions, which was significantly higher than that of 0.47 ± 0.02 μg L^{-1} under flooded conditions ($P < 0.05$). The non-flooded treatment showed lower concentrations of total Fe, Mn, and Cu, but higher concentrations of Zn^{2+} and SO_4^{2-} in porewater than the flooded treatment (Table S3). The root HCl extract showed a significantly higher Cd concentration (1.11 ± 0.12 mg kg^{-1}) under non-flooded conditions relative to that of 0.63 ± 0.11 mg kg^{-1} under flooded conditions (Table S1).

The Cd concentrations in rice plants ranked as roots (0.51 ± 0.04 mg kg^{-1}) > stem (0.33 ± 0.02 mg kg^{-1}) > leaves (0.19 ± 0.04 mg kg^{-1}) under non-flooded conditions, and ranked as roots (0.42 ± 0.03 mg kg^{-1}) > stem (0.22 ± 0.00 mg kg^{-1}) ≈ leaves (0.20 ± 0.02 mg kg^{-1}) under flooded conditions (Table S1). The non-flooded treatment had a significantly higher Cd concentration in the roots and stem than the flooded treatment ($P < 0.05$), while both treatments showed similar Cd concentrations in individual leaves, including the fifth, fourth, third, second, and first leaves (Fig. 1a). The dry weights of various rice compartments did not differ significantly between the flooded and non-flooded treatments, except that the roots in the non-flooded treatment yielded a significantly lower dry weight than that in the flooded treatment ($P < 0.05$) (Fig. 1b). Consequently, no significant difference in Cd mass was observed in the roots or individual leaves between the two treatments, while a significantly higher Cd mass was found in the stem in the non-flooded treatment than in the flooded treatment ($P < 0.05$) (Fig. 1c). The translocation factor of Cd from roots to shoots was similar under flooded and non-flooded conditions ($TF_{\text{shoot/root}} = 0.41–0.44$). The Cd concentrations in individual leaves decreased from 0.24–0.26 mg kg^{-1} to 0.10–0.12 mg kg^{-1} in sequence from the fifth leaf to the first leaf, but a reverse result was observed for the dry weights in both treatments. In particular, the dry weights of the first leaf were higher than those of the other leaves, which resulted in a higher Cd mass in the first leaf than in the other leaves.

3.2. Cadmium isotope fractionation

The Cd isotopes fractionated in a positive direction from bulk soil to porewater under flooded and non-flooded conditions, indicating that the Cd isotope composition in the porewater was heavier than that in the bulk soil (Fig. 2). The root HCl extracts had lighter Cd isotopes than the porewater and rice roots, and the $\delta^{114/110}Cd$ values of root HCl extracts were similar in the two treatments. A negative fractionation from porewater to the whole rice was found in the flooded ($\Delta^{114/110}Cd_{\text{whole rice-soil porewater}} = -0.15 \pm 0.06‰$) and non-flooded treatments ($\Delta^{114/110}Cd_{\text{whole rice-soil porewater}} = -0.39 \pm 0.06‰$). The flooded and non-flooded treatments displayed a reverse fractionation direction from roots to shoots (flooded: $\Delta^{114/110}Cd_{\text{shoot-root}} = 0.19 \pm 0.03‰$, non-flooded: $\Delta^{114/110}Cd_{\text{shoot-root}} = -0.67 \pm 0.06‰$). The total leaf showed isotopically heavier Cd than the stem under both conditions (flooded: $\Delta^{114/110}Cd_{\text{leaf-stem}} = 1.01 \pm 0.10‰$, non-flooded: $\Delta^{114/110}Cd_{\text{leaf-stem}} = 0.77 \pm 0.08‰$). The Cd isotopic compositions of the fourth leaf were heavier than those of the fifth leaf in the flooded and non-flooded treatments, whereas the Cd isotopes fractionated in the opposite direction from the fourth leaf to the second leaf in sequence. Eventually, the first leaf in the two treatments shifted negatively relative to the second leaf and showed a $\delta^{114/110}Cd$ value similar to that of the fifth leaf.

3.3. Gene expression quantification

The expression levels of *OsNRAMP1* and *OsNRAMP5* in roots were significantly higher in the non-flooded treatment than in the flooded treatment (Fig. 3a). Generally, *OsGS1* and *OsPCS1* genes showed distinct expression patterns between the non-flooded and flooded treatments, in which the non-flooded treatment showed a lower expression level of *OsGS1* in most of the tested tissues, but higher expression of *OsPCS1* in all tissues (Fig. 3b and c). The differences in the relative expression

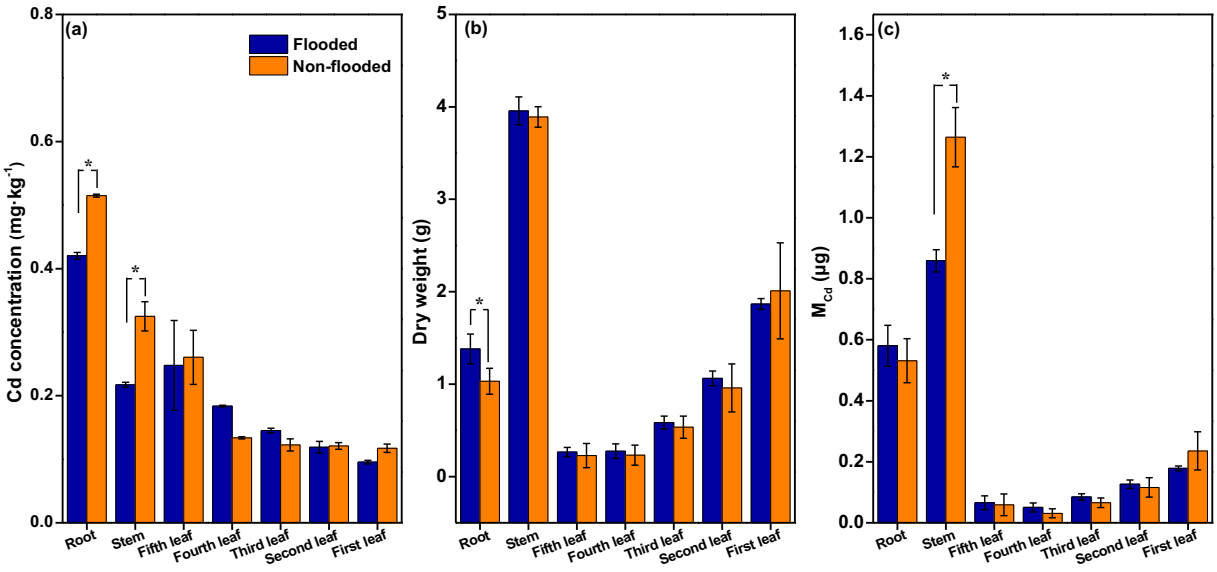

Fig. 1. (a) Cadmium concentration, (b) dry weight, and (c) Cd mass in various rice compartments under flooded and non-flooded conditions at jointing stage. Values are means ± sd of three individual replicates of pots, and the significant differences determined with independent-sample *t*-test are indicated by * ($P < 0.05$).

of *OsPCS1* between the two treatments were more significant than those of *OsGS1*, and all the leaves had higher expression levels of *OsPCS1* than the roots and stems. For the *OsHMA3* gene, its expression levels in the fifth, fourth, and third leaves were higher in the non-flooded treatment than those in the flooded treatment, but reverse results were observed in the stems, second leaf, and flag leaf (Fig. 3d). The expression of both *OsHMA2* and *CAL1* genes in roots and stems was upregulated in the non-flooded treatment relative to the flooded treatment (Fig. 3e). It should be noted that the relative expression levels of *CAL1* were substantially higher than those of *OsHMA2* in the non-flooded treatment. The *OsMT1e* gene expression levels in the fifth, fourth, and third leaves were significantly higher than those in other tissues, and the non-flooded treatment showed significantly higher expression of *OsMT1e* in the fifth, fourth, and third leaves relative to the flooded treatment (Fig. 3f).

4. Discussions

4.1. Cadmium isotope fractionation during uptake by root

The negative fractionation of Cd isotopes from porewater to the whole rice ($\Delta^{114/110}Cd_{\text{whole rice-soil porewater}} = -0.39$ to -0.15‰) is similar to the range of -0.34 to -0.06‰ in previous studies (Imseng et al., 2019; Wiggenhauser et al., 2021a). These results indicate that rice plants preferentially absorb light Cd isotopes from soil porewater, which may be influenced by Cd transfer in soil and absorption into

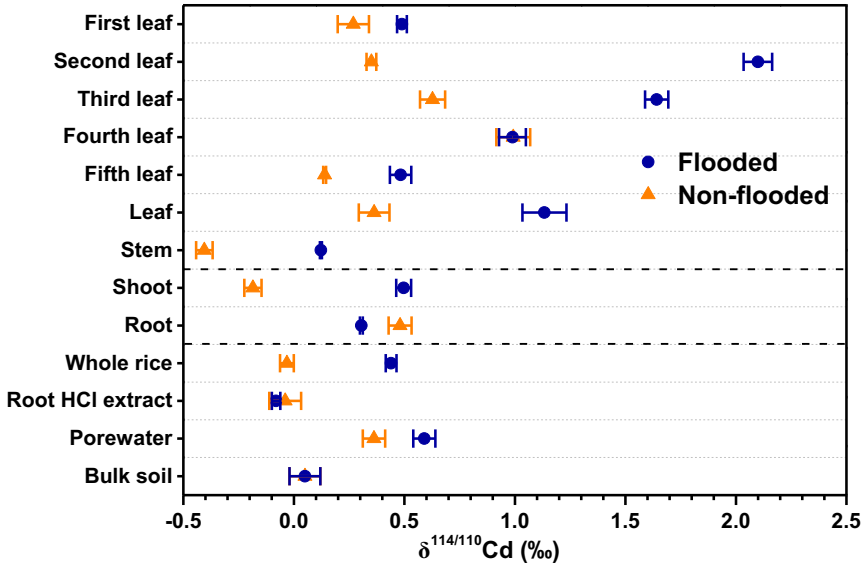

Fig. 2. Cd isotope compositions in bulk soil, soil porewater, root HCl extract, whole rice plant, root, shoot, stem, total leaf and individual leaves under flooded and non-flooded conditions at jointing stage. Samples from three individual pots were combined and analyzed in triplicate for Cd isotope composition and the $\delta^{114/110}$Cd values are presented relative to the standard reference of NIST SRM 3108 with the error bars denoting ±2sd ($n = 3$). The $\delta^{114/110}$Cd values and the corresponding 2sd values (means ±2sd) for the whole rice plants, shoots and total leaves were calculated using the mass balance and error propagation following Eq. (3) and Eqs. (4)–(6), respectively. The $\delta^{114/110}$Cd values of an experimental replicate of root HCl extract, root and the third leaf under flooded conditions, and of root HCl extract under non-flooded conditions were − 0.11 ± 0.03‰, 0.29 ± 0.02‰, 1.72 ± 0.03‰ and 0.02 ± 0.06‰, respectively.

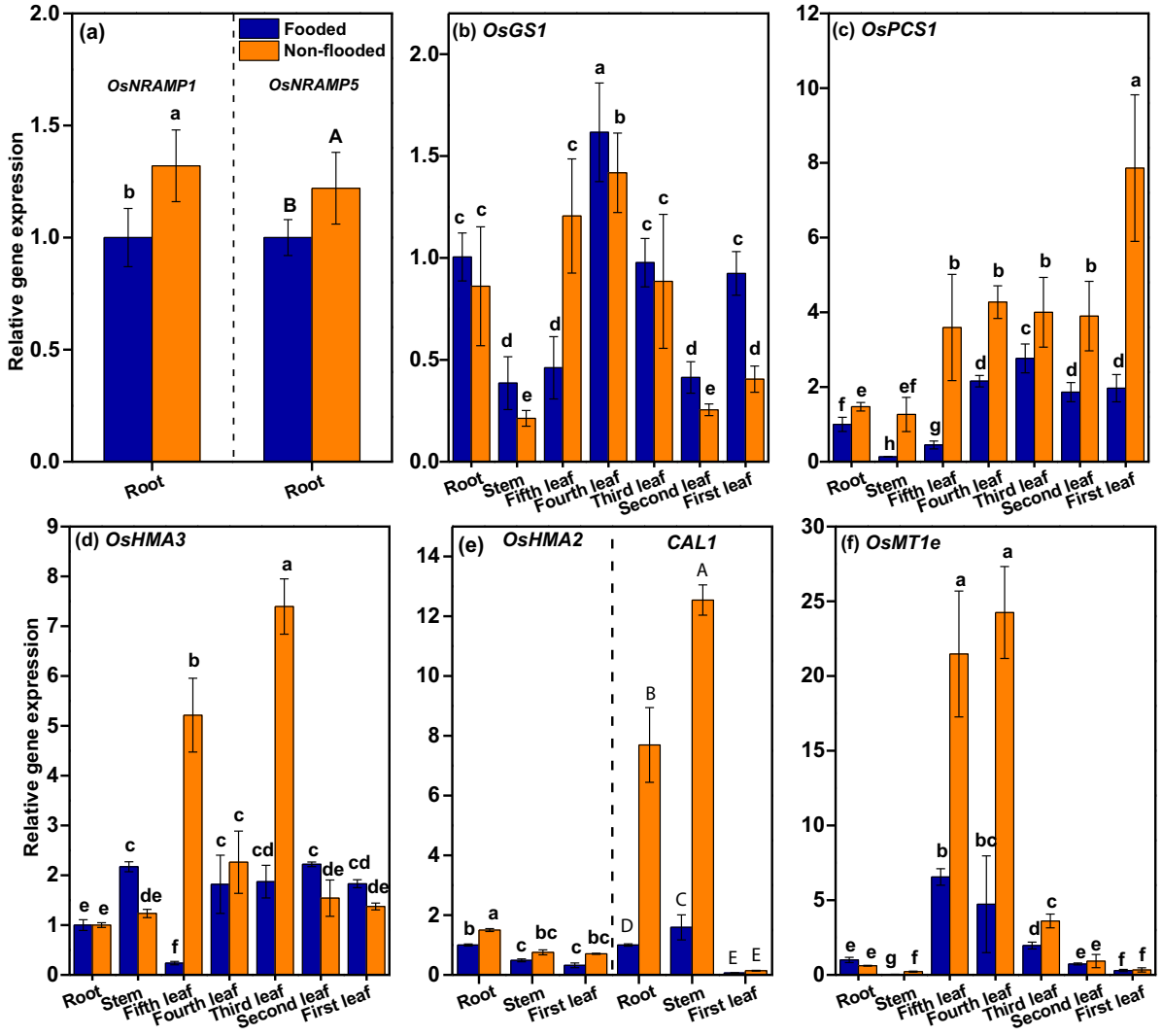

Fig. 3. The relative expression levels of genes related to Cd transport (a) *OsNRAMP1* and *OsNRAMP5*, (b) *OsGS1*, (c) *OsPCS1*, (d) *OsHMA3*, (e) *OsHMA2* and *CAL1*, and (f) *OsMT1e* in roots, stems and individual leaves under flooded and non-flooded conditions at jointing stage. The expression in the roots under flooded conditions was used as the control for comparison of the results obtained for individual genes. Values are means ± sd of three individual replicates of pots. The letters indicate significant differences calculated with independent-sample *t*-test at the $P < 0.05$ level.

root cells via transporters (Imseng et al., 2019; Wei et al., 2018). First, heavy Cd isotopes are preferentially enriched in porewater when ionic Cd is released from bulk soil, because aqueous Cd species, including free and hydrated Cd^{2+} ions, are bound to the neighboring oxygen atoms and have shorter bonds than those associated with soil (Wiederhold, 2015; Wiggenhauser et al., 2021a). Free Cd^{2+} and Cd-DOM are normally the major forms of Cd in porewater, and the proportion of free Cd^{2+} (plant available) to the total Cd concentrations typically increases with decreasing pH (Imseng et al., 2019; Wiggenhauser et al., 2021a). The non-flooded treatment showed a slightly lower pH value and DOM concentration (pH 7.01, DOM 48.8 mg L^{-1}) than the non-flooded treatment (pH 7.08, DOM 68.1 mg L^{-1}) (Table S3). The proportion of free Cd^{2+} in the porewater of the non-flooded treatment was calculated to be higher than that in the flooded treatment (Fig. S3). In addition, more Cd^{2+} was released in the porewater of the non-flooded treatment than in the flooded treatment (Fig. S2). These results implied that more Cd^{2+} was available for uptake by rice under non-flooded conditions. As a result, the Cd mass in the whole rice of the non-flooded treatment (2.30 μg) was significantly higher than that of the flooded treatment (1.94 μg) (Table S1). It was recently reported that light Cd isotopes were preferentially bound to humic acid relative to aqueous Cd species in solution ($\Delta^{114/110}Cd_{HA\text{-}Cd(aq)} = -0.15‰$) (Ratié et al., 2021). Our flooded treatment showed a higher proportion of Cd-DOM in the porewater than in the non-flooded treatment (Fig. S3), which may contribute to its less negative fractionation from porewater to rice because of the buffering effect of Cd-organic complexes with light isotopes in aqueous solution (Ratié et al., 2021).

Cd^{2+} in the porewater can subsequently diffuse to the root surface for Cd uptake by the roots (Imseng et al., 2019). The observation that diffusion can cause variations in zinc isotopic compositions implies that lighter isotopes could potentially be enriched by diffusion through biological membranes or the boundary layer surrounding reactive particles in aquatic environments (Arnold et al., 2010; Rodushkin et al., 2004). Therefore, such a diffusion effect may also apply for Cd isotope fractionation in soil porewater, although there is no experimental evidence that the diffusion effect is significant for Cd isotopes.

Fe plaque on the root surface may play an important role in affecting Cd uptake by the roots (Huang et al., 2019). The root HCl extracts were preferentially enriched in light Cd isotopes compared to the porewater ($\Delta^{114/110}Cd_{root\ HCl\ extract\text{-}porewater} = -0.40$ to $-0.67‰$) (Fig. 2), and a similar negative fractionation of Cd isotopes was also reported from nutrient solution to the root surface of rice seeding ($\Delta^{114/110}Cd_{root\ extract\text{-}nutrient\ solution} = -0.17‰$) in a recent study (Zhang et al., 2021). Fe plaque formation was clearly observed on the root surface under

flooded and non-flooded conditions (Fig. S4). As such, Fe plaques can serve as scavengers of light Cd isotopes via adsorption and co-precipitation when Cd is diffused to the root surface (Wiggenhauser et al., 2021a; Wasylenki et al., 2014). Under non-flooded conditions, the higher concentration of Cd in porewater could drive more Cd being associated with the Fe plaques (Fig. 1a), but the root HCl extracts showed similar Cd isotope compositions between the non-flooded and flooded treatments (Fig. 2). It has been found that the enrichment of light isotopes in the root extract from soil solution was larger in the non-flooded than in the flooded soils, in which the paddy soil was spiked with Cd to obtain a bulk soil Cd concentration of 15 mg kg^{-1} (Wiggenhauser et al., 2021a). Such a high level of Cd contamination in soil resulted in Cd concentrations in porewater that were four orders of magnitude higher than those in this study. The Cd concentrations in porewater in the non-flooded soil were two to three magnitudes higher than those in the flooded soil in this previous study, which could partly contribute to the larger extent of fractionation in the non-flooded soil (Wiggenhauser et al., 2021a). In this study, the Cd concentrations in porewater under non-flooded conditions were within the same magnitude as those under flooded conditions, and the whole rice represented a larger pool of Cd than the root HCl extracts, resulting in a minor effect on the isotope fractionation of root HCl extracts. In addition, the root surface extracted using $CaCl_2$ in a previous study mainly contained weakly bound Cd (Wiggenhauser et al., 2021a), while that extracted using HCl in this study includes both weakly and strongly bound Cd (Aucour et al., 2015, 2017). These two pools of root extracts are not equivalent and may result in different behaviors in Cd isotope fractionation.

The OsNRAMP5 transporter represents the major pathway for Cd entry into rice roots, although other transporters such as OsNRAMP1 can also mediate the absorption process (Sasaki et al., 2012; Takahashi et al., 2011). The non-flooded treatment revealed an up-regulated expression of both *NRAMP* genes and more pronounced negative fractionation of Cd isotopes from porewater to the whole rice than the flooded treatment. Recently, the NRAMP5 transporter was found to facilitate the uptake of light Cd isotopes in yeast cells transformed with the *TcNRAMP5* gene from *Theobroma cacao* (Moore et al., 2020). Studies have revealed that the methionine sulfur in the metal-binding site of NRAMP preferentially coordinates the Cd substrate (Bozzi et al., 2016). The enrichment of light Cd isotopes in whole rice could be due to an equilibrium fractionation that prefers light isotopes during binding of Cd to these membrane transporters and/or a kinetic fractionation that prefers light isotopes when Cd crosses the membrane transporters (Wasylenki et al., 2014; Zhao et al., 2021). In this study, both *OsNRAMP5* and *OsNRAMP1* genes in the roots were upregulated in the non-flooded treatment relative to the flooded treatment (Fig. 3a), which may be due to the low availability of Mn or Fe in soil porewater (Table S3) (Chang et al., 2020; Sasaki et al., 2012). Since the non-flooded treatment had a higher Cd concentration in the soil porewater (Fig. S2), more Cd could be inadvertently absorbed by roots under non-flooded conditions than under flooded conditions (Fig. 1c). Wiggenhauser et al. (2021a) found that Cd speciation in the bulk soil changed from Cd—O to Cd—S during flooding. The SO_4^{2-} concentration in the soil porewater of the flooded treatment (88.3 mg L^{-1}) was significantly lower than that of the non-flooded treatment (769 mg L^{-1}) (Table S3). Such a decrease in sulfate indicated that sulfate was reduced to sulfides, and part of the Cd also precipitated as Cd sulfide (Fulda et al., 2013). Therefore, more light Cd isotopes were supposed to be preferentially immobilized as Cd—S in the flooded soil (Guinoiseau et al., 2018; Yang et al., 2015), with more heavy isotopes being transferred from soil to plant. This could be one of the reasons why the whole rice in the flooded treatment enriched heavier Cd isotopes than that in the non-flooded treatment (Fig. 2). Considered together, the enrichment of lighter Cd isotopes in the whole rice under non-flooded conditions was ascribed to the higher supply of light Cd isotopes in porewater and the up-regulation of *OsNRAMP5* and *OsNRAMP1* genes in roots (Fig. 4).

4.2. Cadmium isotope fractionation during root-to-shoot transport

Similar root-to-shoot translocation factors were obtained in both non-flooded and flooded treatments (0.41–0.44), although the non-flooded treatment revealed significantly higher Cd concentrations in roots and shoots and higher Cd mass in shoots than the flooded treatment (Table S1). The Cd isotope fractionated negatively from roots to shoots in the non-flooded treatment ($\Delta^{114/110}Cd_{shoot\text{-}root} = -0.67 \pm 0.06‰$), but in the opposite direction in the flooded treatment ($\Delta^{114/110}Cd_{shoot\text{-}root} = 0.19 \pm 0.03‰$), which indicated that lighter and heavier Cd isotopes were transported from roots to shoots under non-

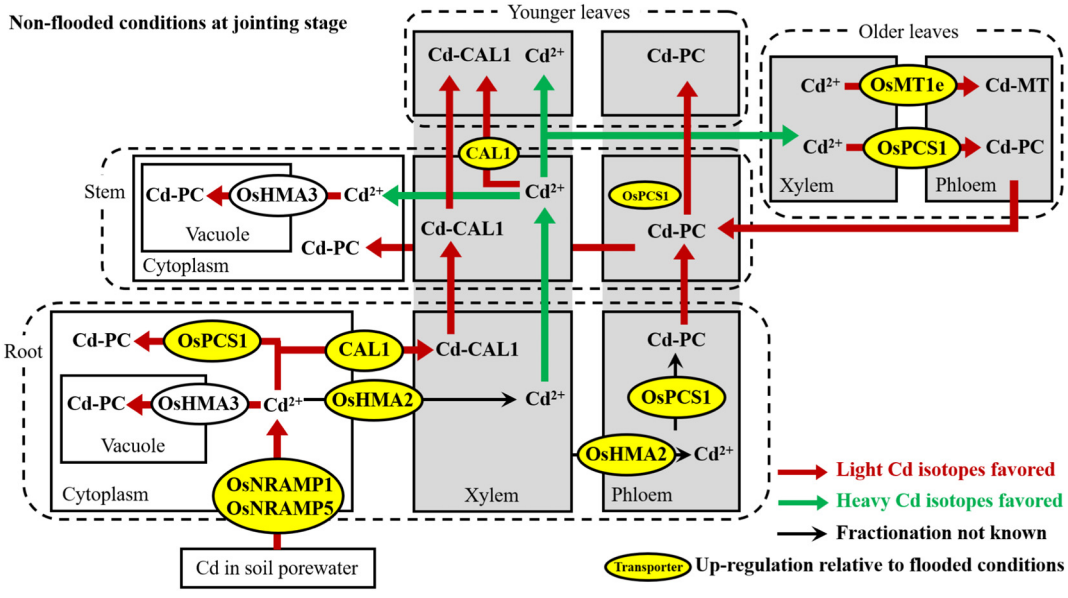

Fig. 4. The main Cd species transported in the rice plant (e.g., Cd^{2+}, Cd-PC, Cd-CAL1, and Cd-MT) and expression of *OsNRAMP1*, *OsNRAMP5*, *OsHMA3*, *OsHMA2*, *CAL1*, *OsPCS1*, and *OsMT1e* genes during root uptake, root-to-shoot and stem-to-leaf transport under non-flooded conditions at jointing stage are proposed based on the results of Cd isotopic fractionation and gene expression in Figs. 2 and 3. Cd-PC, Cd-CAL1, and Cd-MT are considered as the isotopically light Cd species, while Cd^{2+} as the isotopically heavy Cd species. As presented in Fig. S1, the first, second, third, fourth and fifth leaves from top to bottom are divided into two groups: the first, second, and third leaves as younger leaves and the fourth and fifth leaves as older leaves.

flooded and flooded conditions, respectively. Within cereals, Cd isotopes are usually observed to fractionate positively from roots to shoots/straw in previous reports (rice with functional OsHMA3: $\Delta^{114/110}Cd_{shoot\text{-}root} = 0.16$ to 0.19‰; Wheat and barley: $\Delta^{114/110}Cd_{straws\text{-}root} = 0.21$ to 0.41‰) (Imseng et al., 2019; Wiggenhauser et al., 2016, 2021a). The OsHMA3 transporter plays an important role in sequestering Cd into the root vacuoles, in which chelating Cd—S complexes are the major form of Cd with lighter isotopes, leaving heavier isotopes such as Cd^{2+} and Cd—O complexes transported to the shoots (Wiggenhauser et al., 2021a; Zhang et al., 2021). This can also explain the positive fractionation of Cd isotopes during root-to-shoot transfer in our flooded treatment.

In the non-flooded treatment, however, the expression level of *OsHMA3* gene in roots was similar to that in the flooded treatment, and that of *OsHMA2* gene in roots was up-regulated compared to the flooded treatment (Fig. 3d and e), which contradicts our expectation. When rice seedlings were exposed to Cd in the hydroponic solution (≥ 0.1 μM Cd^{2+}), the transcriptions of *OsHMA3* and *OsHMA2* in roots were neither influenced by rising Cd exposure nor related to the changes in Cd root retention (Nocito et al., 2011). The expression of *OsHMA2* in the roots of other rice cultivars increased under iron deficiency (Takahashi et al., 2012). Therefore, the significantly lower Fe concentration in the porewater of the non-flooded treatment compared to the flooded treatment (Table S3) may upregulate the expression of *OsHMA2* in the roots. In this study, the samples were collected at the jointing stage of rice growth, during which the plants demand the highest level of nutrients for stem elongation and leaf development (Kashiwagi et al., 2009; Yamaji and Ma, 2014). OsHMA2 functions as a Cd and zinc transporter from the apoplast to the symplast to facilitate translocation to the shoot elongation zone via the phloem (Yamaji et al., 2013). The higher Cd accumulation in the shoots than in the roots under non-flooded conditions could be associated with the upregulated expression of *OsHMA2* in the roots, which facilitated the loading of Cd species such as free Cd^{2+} and Cd-ligand complexes into xylem and phloem for upward transport (Figs. 1c & 4). However, the expression of *OsHMA2* alone could not explain the markedly negative fractionation from root to shoot under non-flooded conditions, since Cd sequestration by TcHMA proteins has been found to be associated with a fractionation within the range of −0.24 to 0.21‰ (Moore et al., 2020).

The expression of *CAL1* in roots was substantially higher in the non-flooded treatment (Fig. 3e), which implied that a large portion of Cd in roots should be complexed with CAL1 in the xylem parenchyma cells and then transported to the shoot via the xylem. Cd is supposed to be coordinated to three thiol groups in CAL1 to form a stable Cd:3(SH−) complex (Luo et al., 2018; Zhao and Huang, 2018). The equilibrium isotope fractionation between a hydrated Cd species and a Cd bound to a sulfur chelator may result in the enrichment of light isotopes in the sulfur-bound Cd species (Zhao et al., 2021). Although CAL1 is postulated to contribute to only 20% of the Cd translocation in rice seedlings exposed to Cd (Luo et al., 2018), the significant upregulation of *CAL1* expression in roots is supposed to play an important role in facilitating enrichment of lighter Cd isotopes in shoots under non-flooded conditions, particularly when the shoots accumulated a higher Cd mass than the roots (Fig. 4). In addition, the opposite direction of isotope fractionation from roots to shoots between the non-flooded and flooded treatments was more likely controlled by the CAL1 ligand, since the expression level of *CAL1* in roots was substantially higher than that of *OsHMA3* and *OsHMA2* under non-flooded conditions (Fig. 3).

4.3. Cadmium isotope fractionation between stems and leaves

The positive fractionation of Cd isotopes from stems to leaves under both non-flooded and flooded conditions demonstrated that the leaves were preferentially enriched in heavy Cd isotopes compared to the stems (Fig. 4). Previous studies mainly focused on Cd isotope fractionation from roots to shoots/straw but did not differentiate the shoots into stems and leaves in cereals, including wheat, barley, and rice (Imseng et al., 2019; Wiggenhauser et al., 2016, 2021a). Recently, Moore et al. (2020) reported that the leaves of cacao seedlings showed an enrichment of isotopically heavy Cd relative to the whole plants, which is in accordance with our observations with rice.

The expression of the *CAL1* gene was also observed in the stems under both non-flooded and flooded conditions (Fig. 3e), which is likely due to its preferential expression in the leaf sheath of rice (Luo et al., 2018). No detectable expression of *CAL1* was found in the fifth, fourth, third, or second leaves in our experiments, which is consistent with a previous finding that *CAL1* transcript expression was almost undetectable in the leaf blade (Luo et al., 2018). Previous studies detected CAL1/Cd-CAL1 in the leaf blade, which is considered to be extruded from xylem parenchyma cells and/or leaf sheath into xylem sap and subjected to long-distance transport towards the leaf blades (Hussain et al., 2021; Luo et al., 2018). The significantly higher expression of *CAL1* in stems under non-flooded conditions likely facilitated the formation of Cd-CAL1 complexes in the xylem of stems. The Cd-CAL1 complexes are supposed to be enriched in lighter isotopes compared to free and hydrated Cd^{2+}, based on the fact that Cd chelated with S ligands prefers light Cd isotopes during equilibrium fractionation (Zhao et al., 2021). Luo et al. (2018) observed that CAL1 did not affect Cd accumulation in rice grains but increased Cd accumulation in xylem sap and leaves, and assumed that the Cd-CAL1 complexes could not be taken back into symplastic compartments. If this is the case, more Cd-CAL1 with light isotopes could be immobilized in the xylem/apoplast of stems and leaves, which contributed to the lighter Cd isotopes in the stems and leaves in the non-flooded treatment than in the flooded treatment.

The complexation of Cd with PCs and its precursor GSH is another detoxification process in plants under Cd stress (Park et al., 2017). The expression levels of *OsPCS1* in stems and leaves were relatively higher than those of *OsGS1* under both non-flooded and flooded conditions, which suggested that Cd preferentially complexed with PCs in the shoots (Fig. 3b and c). The non-flooded treatment had a higher expression of *OsPCS1* but a lower expression of *OsGS1* in the stems and leaves, which is in accordance with previous observations that PCs were increased but GSH was decreased under higher Cd stress (Liu et al., 2015; Yan et al., 2016). An enrichment of light towards heavy Cd isotopes can be expected in the order of Cd—S donors in PC < Cd-S donors in GSH < Cd-O donors according to theoretical calculations using model organic ligands (Zhao et al., 2021). The significantly higher expression of *OsPCS1* in the stems and leaves of non-flooded treatment could facilitate the formation of Cd-PC complexes that prefer lighter isotopes, which could also contribute to the enrichment of lighter isotopes in these organs of non-flooded treatment relative to the flooded treatment.

4.4. Cadmium isotope fractionation among leaves

The expression of *OsMIT1e* in the different leaves followed a descending order from the fifth leaf to the first leaf (Fig. 3f), suggesting that Cd in older leaves might preferentially bind to metallothioneins. Metallothioneins in plants normally have a high percentage of cysteine, a thiol-containing amino acid, and can form metal-thiolate clusters for detoxification of heavy metals (Malekzadeh and Shahpiri, 2017; Samuel et al., 2021). If this process dominates, it could favor the immobilization of Cd with lighter isotopes in older leaves than in younger leaves (Rono et al., 2021). This supports the finding that the fifth leaf enriched lighter Cd isotopes than the fourth leaf under both conditions. However, Cd isotopes fractionated in the opposite direction from the fourth to third and second leaves in the non-flooded and flooded treatments, which may be controlled by processes other than metallothionein immobilization.

The highest Cd accumulation was found in the youngest leaves because the youngest leaves showed the highest biomass, although their Cd concentrations were lower than those in the older leaves (Fig. 1c).

This is consistent with previous findings that Cd is preferentially transported towards the newest leaf due to a higher transpiration rate and greater demand for nutrients for leaf development in young leaves (Page and Feller, 2015; Kobayashi et al., 2013). Free Cd^{2+} is reported to be the major transport form in the xylem sap (Alvarez-Fernández et al., 2014; Clemens and Ma, 2016). The increasing enrichment of heavier Cd isotopes from the bottom leaf to the second leaf under flooded conditions may be partially ascribed to the Cd^{2+} transported to the upper leaves via the xylem (Li et al., 2019). In addition, Cd in the xylem of stems can be immediately loaded onto the phloem after Cd is transported from the root, and Cd may bind to thiol-containing ligands such as GSH and PCs in the phloem sap of rice (Kato et al., 2010; Kobayashi et al., 2013; Yamaji and Ma, 2014). Such an intervascular transfer process can be mediated by OsHMA2 (Yamaji et al., 2013), but no resolvable isotope fractionation has been observed in transgenic yeast expressing *TcHMA2* from the cacao plant (Moore et al., 2020). The substantially higher expression of *CAL1* than *OsHMA2* in stems suggests that CAL1 protein plays a more important role in Cd distribution between stems and leaves. In addition, Cd-PCs were likely another major form of Cd transported via phloem to and accumulated in the younger leaves under non-flooded conditions (Yoneyama et al., 2015), in which the expression levels of *OsPCS1* in the stems and leaves were significantly upregulated relative to the flooded conditions. It is intriguing to find that the Cd isotopes in the first leaf were similar to those in the fifth leaf under both conditions, which implied that the Cd from the fifth leaf might be an important source of Cd transported to the first leaf via the phloem.

5. Conclusion

The current study demonstrated that linking the Cd isotope signature of different rice compartments to the gene expression of membrane transporters and complexed proteins/peptides that are involved in Cd transport can provide an improved understanding of the key processes that govern the transfer of Cd in soil-rice systems. Our results confirmed that rice plants preferentially absorb lighter Cd isotopes via OsNRAMP1 and OsNRAMP5 transporters, and such enrichment can be more pronounced when more Cd is available in soil porewater, i.e., under non-flooded conditions. Vacuolar sequestration via OsHMA3 resulted in lighter Cd isotopes in the roots when rice was grown in flooded soils. Within the shoots, the majority of Cd was detected in the stems, which was preferentially enriched in lighter isotopes than in the leaves. This could be ascribed to the higher expression of *OsPCS1* and *OsHMA3* in the stems, which favor the enrichment of Cd-PCs with lighter isotopes, leaving heavier isotopes being transported to the leaves. Cd in the older leaves might preferentially bind to metallothioneins like OsMIT1e, but this process only showed limited contribution to restricting Cd mobility. The Cd species with lighter isotopes such as Cd-PCs were readily transported from the stem and older leaves to younger leaves, which was more pronounced under non-flooded conditions.

To the best of our knowledge, this study is the first attempt to apply the combination of Cd isotope technique together with gene expression to explore the key processes controlling Cd transport in rice plants. Although challenges and limitations remain during the interpretation of data, the findings obtained in this study are of critical importance in developing control strategies during the jointing stage for the safe production of rice in Cd-contaminated paddy soils. It is noteworthy that the shoots were preferentially enriched in lighter Cd isotopes relative to the roots under non-flooded conditions, which is different from the positive fractionation observed in our flooded treatment and in previous studies (Imseng et al., 2019; Wiggenhauser et al., 2016, 2021a). The upregulated expression of the *CAL1* gene was more pronounced than that of the *OsHMA3* gene in the roots under non-flooded conditions. Together, these isotopic and genetic results suggest that xylem loading via CAL1 plays the most important role in facilitating the root-to-shoot transport of isotopically lighter Cd when more Cd is absorbed by roots under non-flooded conditions. As such, a strategy that facilitates Cd-CAL1 accumulation in the xylem would reduce Cd loading into the phloem for grain filling. Further investigations are required to determine which processes mainly control the upward translocation of Cd to rice grains at the grain-filling stage, in particular, how Cd accumulation in stems contributes to Cd accumulation in grains via nodes.

CRediT authorship contribution statement

Songxiong Zhong: Formal analysis, Investigation, Visualization, Writing – original draft. **Xiaomin Li:** Supervision, Visualization, Data curation, Writing – original draft. **Fangbai Li:** Conceptualization, Supervision, Writing – review & editing. **Yingmei Huang:** Investigation. **Tongxu Liu:** Writing – review & editing, Project administration. **Haoming Yin:** Methodology, Validation. **Jiangtao Qiao:** Investigation. **Guojun Chen:** Writing – review & editing. **Fang Huang:** Methodology, Resources.

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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.150633.

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