Supplementary Information to:

Cadmium isotope fractionation reveals genetic variation in Cd uptake and translocation by *Theobroma cacao* and role of natural resistance-associated macrophage protein 5 and Heavy Metal ATPase-family transporters

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Table S1. Comparison of Cd concentrations and isotope compositions obtained for reference and quality control materials and comparison with literature data and results obtained for duplicate digests and analyses of roots and leaf samples from a single Matina 1-7 plant.

Material	Type	n	[Cd] ± sd ($\mu g\ g^{-1}$)	[Cd] Lit. ($\mu g\ g^{-1}$)	$\delta^{114/110}Cd$ ± 2sd (‰)	$\delta^{114/110}Cd$ Lit. (‰)
BAM-I012[a]	Cd solution	16	--	--	–1.32±0.06	–1.33±0.04
NIST SRM 1570a[b]	Spinach leaf	4	2.709±0.01	2.876±0.058	0.44±0.06	--
IAEA-359[c]	Cabbage leaf	1	0.123	0.125±0.005	--	--
MAGIC NA-702[d]	Cacao leaf	10	107±18	102	–0.55±0.13	–0.50±0.06
Sigma Aldrich $CdCl_2$[e]	Cd additive	7	--	--	–0.36±0.04	--
Matina 1-7 Digest a	Cacao root	1	195	--	–0.71±0.05	--
Matina 1-7 Digest b	Cacao root	1	218	--	–0.65±0.07	--
Matina 1-7 Digest a	Cacao leaf	1	3.79	--	–0.18±0.06	--
Matina 1-7 Digest b	Cacao leaf	1	2.96	--	–0.23±0.05	--

n = number of individual samples that were digested and analysed. [a] The literature data are the mean result from Abouchami *et al.*, 2013.[1] [b] Cd concentration from certificate. No published $\delta^{114/110}$Cd data are available for this sample. [c] The published Cd result is an 'Information value' from the certificate that is based on results from 57 laboratories. [d] In-house quality control material prepared from cacao leaves, whereby the plant was grown in a hydroponic solution containing added Cd; the literature results for this sample are from Barraza *et al.*, 2019.[2] [e] Source of Cd that was added to the hydroponic solutions and the yeast culture medium.

Table S2: Weights of the plant tissue samples

Cacao clones	Leaf g	Root g	Stem g	Total plant g
B 5/7 [POU]	0.554	0.092	0.246	0.892
Catie 1000	0.304	0.127	0.122	0.553
CC 41 (1)	0.463	0.101	0.231	0.795
CC 41 (2)	0.685	0.128	0.189	1.002
CL 19/10	1.361	0.159	0.289	1.809
GU 207/H	0.250	0.092	0.055	0.397
GU 263/V	0.332	0.144	0.089	0.565
IMC 27	0.301	0.132	0.147	0.580
LP 1/41 [POU]	0.635	0.101	0.259	0.995
Matina 1-7	1.387	0.269	0.499	2.155
NA 702	1.181	0.198	0.341	1.720
PNG 340	0.940	0.206	0.551	1.697
POUND 12/A [POU]	0.525	0.121	0.139	0.785
RB46	0.512	0.134	0.140	0.786
RIM 189 [MEX]	1.298	0.299	0.334	1.931
SCA 9	0.668	0.122	0.262	1.053
SPA 9 [COL]	0.535	0.127	0.218	0.880
TARS 31	0.534	0.280	0.181	0.994
TSA 654	0.616	0.138	0.153	0.907
U 70 [PER]	0.247	0.068	0.084	0.399
Mean	**0.666**	**0.152**	**0.226**	**1.045**
sd	**0.361**	**0.064**	**0.127**	**0.514**

Table S3. Name, accession number and donor organization of the cacao germplasm used in the study.

Clone Name	Accession number (ICQC, R)	Donor Collection
B 5/7 [POU]	RUQ 522	ICG, T
CATIE 1000	RUQ 844	CIRAD
CC 41	RUQ 1428	ICG, T
CL 19/10	RUQ 905	ICG, T
GU 207/H	RUQ 225	CIRAD
GU 263 /V	RUQ 1545	CIRAD
IMC 27	RUQ 515	ICG, T
LP 1/41 [POU]	RUQ 353	ICG, T
MATINA 1/7	RUQ 1333	ICG, T
NA 702	RUQ 1587	ICG, T
PNG 340	RUQ 1304	CIRAD
POUND 12/A	RUQ 1458	ICG, T
RB 46 [BRA]	RUQ 134	CATIE
RIM189 [MEX]	RUQ 310	CATIE
SCA 9	RUQ 1064	ICG, T
SPA 9 [COL]	RUQ 235	ICG, T
TARS 31	RUQ 1394	USDA-TARS
TSA 654	RUQ 1029	CEPLAC/CEPEC
U 70 [PER]	RUQ 1488	UNAS

CATIE	Centro Agronómico Tropical de Investigión y Enseñanza (Costa Rica)
CEPLAC	Comissão Executiva do Plano da Lavoura Cacaueira (Brazil)
CIRAD	Centre de cooperation international en recherché agronomique pour le développement (France)
ICG, T	International Cocoa Genebank (Trinidad)
ICQC, R	International Cocoa Quarantine Centre, Reading (UK)
UNAS	Universidad Nacional Agraria de la Selva (Peru)
USDA-TARS	United States Department of Agriculture, Tropical Crops and Germplasm Research, Mayaguez (Puerto Rico)

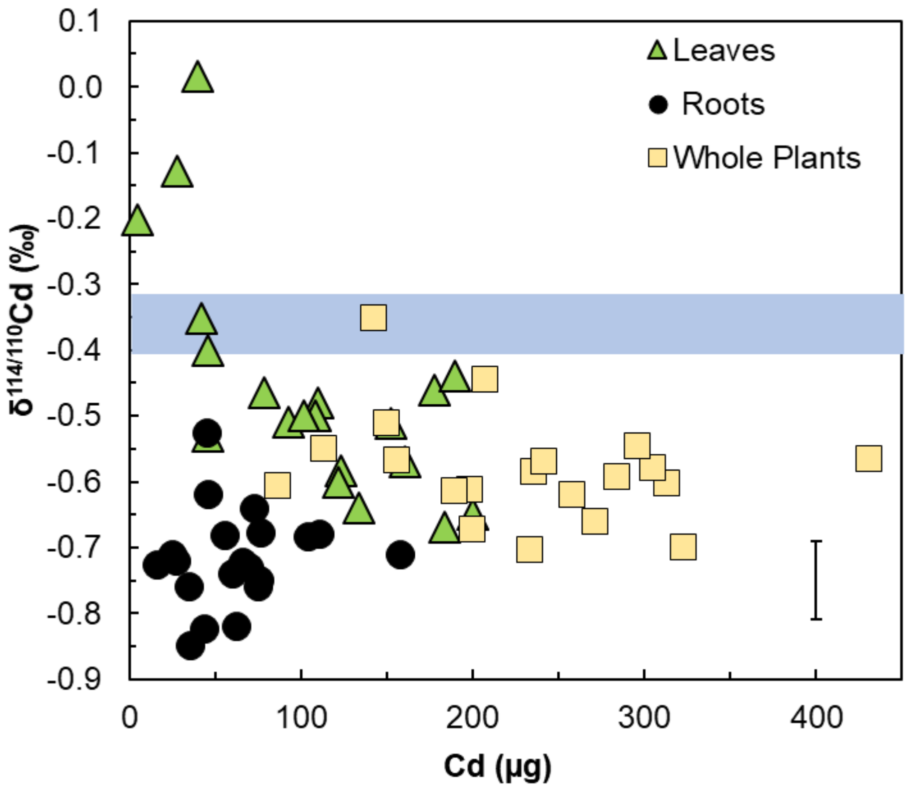

Fig. S1. Plot of $\delta^{114/110}$Cd versus Cd for the leaf, root and whole plant samples of the 19 cacao clones.

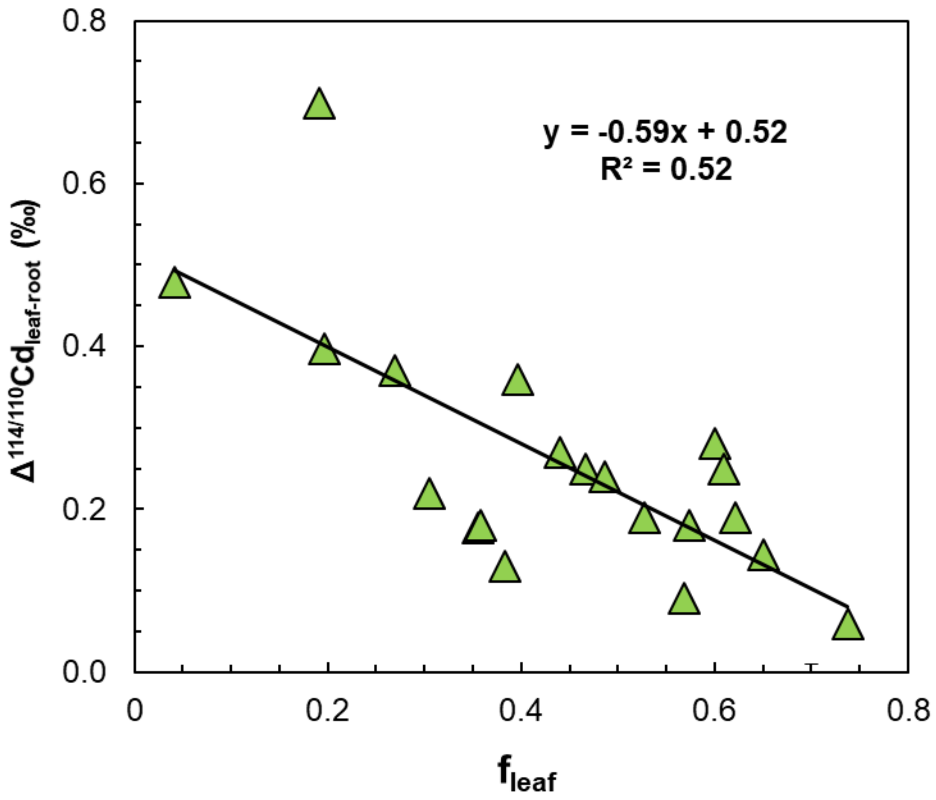

Fig. S2. Plot of $\Delta^{114/110}Cd_{leaf-root}$ versus mass fraction of Cd present in leaves (f_{leaf}) of the 19 analysed cacao clones. A scattered but clear negative correlation is apparent.

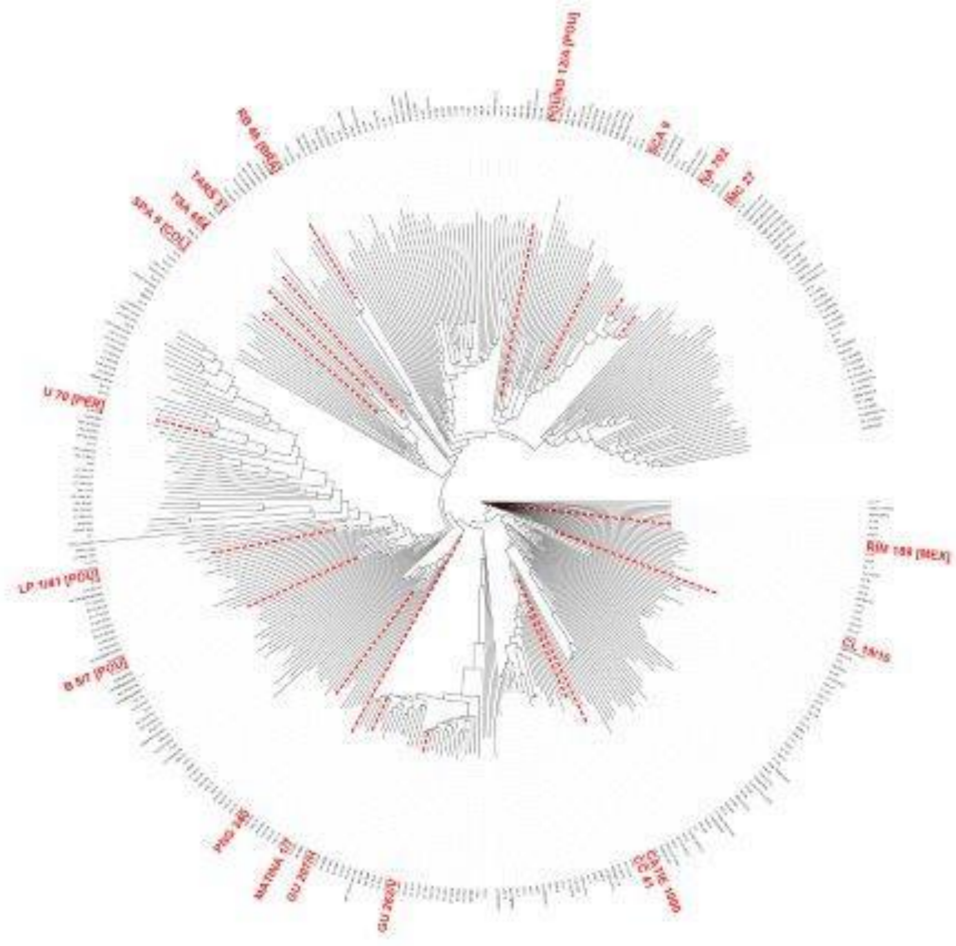

Fig S3. For germplasm selection, a maximum likelihood tree based on the JTT matrix-based model was generated in MEGA7 using the single nucleotide polymorphism (SNP) data from 380 cacao clones. The genetically diverse clones (in red) were selected using information from the evolutionary tree. The SNP data were obtained from the International Cocoa Quarantine Centre, Reading, UK.

(See http://www.icgd.reading.ac.uk/ref_search.php?refcode=ZHA14A).

Note S1: Calculation of Cd inventories for cacao stem and total plants

In this study, the stems of the cacao clones were weighed (alongside roots and leaves; Table S3) but not analysed to determine Cd isotope compositions and concentrations. To enable estimates for the Cd mass and isotope budgets of the total plants, the stems were assigned Cd concentrations $[Cd]_{stem}$ at the midpoint of the results obtained for roots and leaves:

$$[Cd]_{stem} = 0.5\,[Cd]_{root} + 0.5\,[Cd]_{leaf} \qquad \text{(Eq. 1)}$$

The estimated $[Cd]_{stem}$ data were used in conjunction with $[Cd]_{root}$, $[Cd]_{leaf}$ and the weights of the plant organs (Table S3) to determine the mass fractions of Cd present in the roots, stems and leaves (f_{root}, f_{stem}, f_{leaf}). These data were then applied to determine $\delta^{114/110}Cd_{stem}$:

$$\delta^{114/110}Cd_{stem} = f_{root}\,\delta^{114/110}Cd_{root} + f_{leaf}\,\delta^{114/110}Cd_{leaf} \qquad \text{(Eq. 2)}$$

This approach is firmly supported by published Cd data for plants (see below). The estimated $[Cd]_{stem}$ and $\delta^{114/110}Cd_{stem}$ values were subsequently applied to calculate the Cd concentrations, contents and isotope compositions of the total cacao plants.

To obtain uncertainty estimates for the cocoa plant Cd (isotope) mass balances, it was assumed (again in accord with literature data; see below) that deviations from the estimate provided by Eq. 1 are typically evidenced by stem compositions that are more similar to the roots than the leaves. Based on this, the most extreme stem composition that was evaluated to define the maximum ± uncertainty for each clone was thus calculated as:

$$[Cd]_{stem} = 0.75\,[Cd]_{root} + 0.25\,[Cd]_{leaf} \qquad \text{(Eq. 3)}$$

whereby the respective $\delta^{114/110}Cd_{stem}$ value was subsequently determined using Eq. 2. The propagated uncertainty estimates for $\delta^{114/110}Cd_{tot}$ and $\Delta^{114/110}Cd_{leaf\text{-}tot}$ furthermore encompass the analytical errors of the Cd isotope measurements.

Use of the mass balance calculations to estimate the Cd (isotope) inventory of the cacao stems increases the uncertainties for the Cd (isotope) budgets of the total plants. Nonetheless, the approach is useful, reasonable and justified by evidence, practical constraints and the limited impact on the error propagation:

- The reduced number of Cd isotope analyses that are required with the approach significantly reduces the overall analytical cost of the project, as the measurements are associated with considerable cost and staff effort. This was helpful as the resources available for the research were limited.

- Coupled Cd isotope and concentration data that are directly comparable to the results generated in this study are available for cereals wheat, barley[3,4] and Cd accumulators (Cd tolerant *Ricinus communis*, Cd hyperaccumulator *Solanum nigrum*.[5] These literature data were evaluated carefully and used to develop and justify the approach applied here. In detail, the literature results show that the [Cd] and $\delta^{114/110}$Cd values for (i) the straw of wheat/barley are consistently intermediate between the results obtained for roots and grains; (ii) the stems of Cd accumulators are nearly always intermediate between the data for roots and leaves. On average, it is also apparent that the Cd concentrations of the stems/straw of these plants are typically roughly midway between the root and leaf/grain values, whereby most deviations reflect stem results that are somewhat closer to the root rather than the leaf/grain data. As such, the available evidence yields credence to the mass balance approach used here to estimate $[Cd]_{stem}$ and $\delta^{114/110}Cd_{stem}$ and further values derived from these parameters.
- The estimated mass fraction of Cd in the cacao clone stems varies between 15% and 48% with a mean result of f_{stem} = 28 ± 10% (1sd). As such, the stems play an important but not a dominant role in the Cd (isotope) budgets of the cacao clones. Due to this, and even using a conservative estimate for the uncertainty of $[Cd]_{stem}$ and $\delta^{114/110}Cd_{stem}$ (see above), error propagation does not generate unduly large error bars for values such as f_{leaf} and $\Delta^{114/110}Cd_{leaf\text{-}tot}$ (Fig. 3). In fact, the propagated uncertainties are sufficiently small, such that compositional and isotopic differences between the cacao clones are still clearly apparent and can be used to define systematic data trends in Figs. 2, 3 and S2.
- Finally, the validity of the mass balance approach is also demonstrated by the research findings: (i) the measured $\delta^{114/110}$Cd values show similar correlations with $[Cd]_{leaf}$ and Cd_{leaf} (which were both directly measured; Figs. 2a and S1) as well as f_{leaf} (which was calculated; Fig. 2b); (ii) a clear trend is observed for $\Delta^{114/110}Cd_{leaf\text{-}tot}$ (which combines measured and calculated data) with f_{leaf} (Fig. 5). The observed trends are unlikely to be fortuitous but most likely characterise the systematic distribution of Cd (and its isotopes) within the cacao plant, in accord with observations made in previous studies on cereals and Cd accumulators.[3–5]

Note S2: List of the gene sequences used in the study

TcHMA2_ MT151685

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