



Simultaneously decreasing arsenic and cadmium in rice by soil sulfate and limestone amendment under intermittent flooding[☆]

Xu Fang^{a,*}, Andrea E. Colina Blanco^b, Iso Christl^a, Maureen Le Bars^a, Daniel Straub^{c,d}, Sara Kleindienst^{e,f}, Britta Planer-Friedrich^b, Fang-Jie Zhao^g, Andreas Kappler^{d,h}, Ruben Kretzschmar^a

^a Soil Chemistry Group, Institute of Biogeochemistry and Pollutant Dynamics, Department of Environmental Systems Science, CHN, ETH Zurich, CH-8092, Zurich, Switzerland

^b Environmental Geochemistry, Bayreuth Center for Ecology and Environmental Research (BAYCEER), University of Bayreuth, 95440, Bayreuth, Germany

^c Quantitative Biology Center (QBiC), University of Tuebingen, 72076, Tuebingen, Germany

^d Cluster of Excellence: EXC 2124: Controlling Microbes to Fight Infection, Tuebingen, 72076, Germany

^e Microbial Ecology, Department of Geosciences, University of Tuebingen, 72076, Tuebingen, Germany

^f Now: Department of Environmental Microbiology, Institute for Sanitary Engineering, Water Quality and Solid Waste Management (ISWA), University of Stuttgart, Stuttgart, 70569, Germany

^g College of Resources and Environmental Sciences, Nanjing Agricultural University, 210095, Nanjing, China

^h Geomicrobiology, Department of Geosciences, Tuebingen University, 72076, Tuebingen, Germany

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ABSTRACT

Water management in paddy soils can effectively reduce the soil-to-rice grain transfer of either As or Cd, but not of both elements simultaneously due to the higher mobility of As under reducing and Cd under oxidizing soil conditions. Limestone amendment, the common form of liming, is well known for decreasing Cd accumulation in rice grown on acidic soils. Sulfate amendment was suggested to effectively decrease As accumulation in rice, especially under intermittent soil flooding. To study the unknown effects of combined sulfate and limestone amendment under intermittent flooding for simultaneously decreasing As and Cd in rice, we performed a pot experiment using an acidic sandy loam paddy soil. We also included a clay loam paddy soil to study the role of soil texture in low-As rice production under intermittent flooding. We found that liming not only decreased rice Cd concentrations but also greatly decreased dimethylarsenate (DMA) accumulation in rice. We hypothesize that this is due to suppressed sulfate reduction, As methylation, and As thiolation by liming in the sulfate-amended soil and a higher share of deprotonated DMA at higher pH which is taken up less readily than protonated DMA. Decreased gene abundance of potential soil sulfate-reducers by liming further supported our hypothesis. Combined sulfate and limestone amendment to the acidic sandy loam soil produced rice with 43% lower inorganic As, 72% lower DMA, and 68% lower Cd compared to the control soil without amendment. A tradeoff between soil aeration and water availability was observed for the clay loam soil, suggesting difficulties to decrease As in rice while avoiding plant water stress under intermittent flooding in fine-textured soils. Our results suggest that combining sulfate amendment, liming, and intermittent flooding can help to secure rice safety when the presence of both As and Cd in coarse-textured soils is of concern.

1. Introduction

Arsenic (As) and cadmium (Cd) are two non-essential trace elements which can pose severe threats to human health via the consumption of rice (Wang et al., 2019; Li et al., 2011). Compared to other cereals such

as wheat, paddy rice (*Oryza sativa*) is prone to accumulate much higher concentrations of both As and Cd in its grains (Shimbo et al., 2001; Song et al., 2017). On one hand, this is due to the high As and Cd mobility in often acidic (when drained) (Zhu et al., 2016) and reduced (when flooded) paddy soils. On the other hand, rice as a Si- and Mn-

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* Corresponding author.

E-mail address: xu.fang@usys.ethz.ch (X. Fang).

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accumulating plant (Hodson et al., 2005; Sasaki et al., 2012; Yamaji et al., 2013) can efficiently take up As and Cd via Si and Mn transporters (Sui et al., 2018; Su et al., 2010). Inorganic As species (major fractions of As in rice grain) and Cd are both known for high chronic toxicity to humans upon long-term exposure (Hughes et al., 2011; Naujokas et al., 2013), while dimethylarsenate (DMA, another major fraction of As in rice grain) can cause spikelet sterility in rice plants leading to significant yield losses (Tang et al., 2020). Thiolated arsenates (both inorganic and methylated), formed from the reaction of the corresponding oxyarsenates with sulfide and elemental sulfur (Planer-Friedrich et al., 2015; Wallschläger and London, 2008; Kim et al., 2016), have been found to be widely present in paddy soil porewater (also called soil solution) (Wang et al., 2020) and in rice grains (Dai et al., 2022; Colina Blanco et al., 2021). Among the thioarsenates, dimethylmonothioarsenate (DMMTA) has been shown to be highly toxic to *Arabidopsis thaliana* (Pischke et al., 2022) and more cytotoxic than DMA in studies with human cell lines (Moe et al., 2016). However, DMMTA in rice is routinely determined as DMA and, thus, escapes from current regulations, calling for risk assessments regarding the ingestion of DMMTA-containing rice and a corresponding regulation update (Planer-Friedrich et al., 2022).

Various mitigation strategies have been proposed to decrease accumulation of As or Cd in rice grains (Zhao and Wang, 2020). Water management has been shown to be effective in decreasing both As and Cd accumulation in rice but not simultaneously. In fact, adjusting the water regime of rice cultivation always affects As and Cd accumulation in rice in opposite ways, i.e., decreasing one element but increasing the other one (Arao et al., 2009; Honma et al., 2016). Flooding of acidic soils, which are often found in the (sub)tropical rice cultivation regions, limits Cd solubility and rice Cd accumulation mainly by increasing soil pH (Kögel-Knabner et al., 2010). However, soil flooding leads to the reductive dissolution of Fe(III) (oxyhydr)oxide minerals and promotes As methylation, leading to a release of inorganic As (iAs) from soil and an accumulation of iAs and DMA in rice (Arao et al., 2009; Kögel-Knabner et al., 2010; Zhao et al., 2013). Soil drying effectively decreases mobilization of iAs and DMA and their concentrations in rice, but it regenerates soil acidity and favors Cd accumulation in rice (Arao et al., 2009; Kögel-Knabner et al., 2010). Soil amendment by applying limestone or manganese (Mn) to soils has been demonstrated in field trials to decrease Cd concentrations in rice grains grown on acidic soils, especially those low in Mn, by decreasing soil Cd mobility or Cd uptake via Mn–Cd competition, respectively (Fang et al., 2021; Chen et al., 2018). However, effective and practically viable soil amendment strategies for controlling grain As remain unclear.

Suitable combinations of water management with one or multiple soil-amendment materials may offer new possibilities for simultaneously decreasing As and Cd accumulation in rice. In a recent pot study (Fang et al., 2023), the combination of sulfate amendment and intermittent flooding produced rice with lower iAs (−62%) and DMA (−60%) contents, higher yield (+106%), but higher Cd (+365%) compared to the non-sulfate-amended soil under prolonged flooding. Therefore, a combination of sulfate and limestone amendment under an intermittent flooding regime seems to be a promising strategy to simultaneously control grain As and Cd while maintaining good yield. The decrease of grain iAs by sulfate amendment under intermittent flooding was related to the enhanced sulfate reduction in rice rhizosphere, which decreases iron-plaque content and As loading on the rice roots (Fang et al., 2023). Liming-induced changes in pH may also alter microbial activity including dissimilatory Fe(III) and sulfate reduction and, thus, affect the performance of soil sulfate amendment in decreasing As accumulation in rice.

Intermittent flooding, also referred to as alternate wetting and drying (AWD), can support low-As rice production not only by decreasing As mobility in soils but also by boosting soil sulfate reduction (via oxidatively replenishing soil sulfate pool) and its beneficial effects on decreasing grain iAs (Fang et al., 2023). It is also widely recommended

for increasing water-use efficiency (Lampayan et al., 2015; Carrijo et al., 2017), decreasing energy consumption and cost (Lampayan et al., 2015), and decreasing CH₄ emissions during rice cultivation (Liu et al., 2021a; Runkle et al., 2019). However, it may cause plant water stress and yield losses if the soil is dried excessively (Carrijo et al., 2017). Soil texture is known to strongly influence soil aeration (Ben-Noah and Friedman, 2018) and water availability to plants in unsaturated soils (Blume et al., 2016). How soil texture influences the balance between soil aeration, which is needed for decreasing As accumulation in rice, and plant water availability under intermittent flooding and sulfate amendment remains unknown.

Therefore, we designed a pot experiment aiming to investigate the combined effects of soil sulfate and limestone amendment to a sandy loam soil for simultaneously decreasing As and Cd in rice grain. We also compared the sandy loam soil with a clay loam soil under intermittent flooding and sulfate amendment in terms of soil aeration (soil E_h and porewater chemistry), plant water stress (soil matric potential and transpiration rate), and As accumulation and speciation in rice grain. Our study will contribute to a better understanding of interactions among soil sulfate, pH, and texture for securing rice production with low grain As and Cd. Knowledge obtained from this study can also guide the development of mitigation strategies to be tested in future field trials.

2. Materials and methods

2.1. Pot experiment

A pot experiment was carried out in a climate chamber (Kälte 3000) with 12 h/12 h cycles of day (~350 μmol/m²/s light, 28 °C, 60% relative humidity) and night (no light, 23 °C, 75–85% relative humidity). Two acidic paddy soils with different soil texture (USDA texture classification) but similar soil S contents were collected from the Hunan province of China (Table S1): the sandy loam CS soil (Changsha county; 6.7 mg/kg As, 0.51 mg/kg Cd, 302 mg/kg S, 21 mg/kg sulfate-S) and the clay loam HN soil (Hengnan county; 71 mg/kg As, 3.0 mg/kg Cd, 292 mg/kg S, 20 mg/kg sulfate-S). A high-yielding Chinese hybrid rice cultivar, Shenyoun957, which is widely cultivated in southern China, was used in this study. In a previous field trial study, elevated grain As (CS: 0.15–0.46 mg/kg, HN: 0.11–0.47 mg/kg; unpublished data) and Cd concentrations (CS: 0.04–1.27 mg/kg, HN: 0.02–1.85 mg/kg) were observed for this cultivar grown in these two soils (Fang et al., 2021) according to the Chinese regulation limits for iAs (0.35 mg/kg) and Cd (0.2 mg/kg) in brown rice (GB 2762-2022).

Six soil treatments with pot triplicates were designed: CS control (CS), CS + 200 mg/kg sulfate-S supplied as Na₂SO₄ (CS–S), CS + 2308 mg/kg CaCO₃ to increase the soil pH from 5.4 to 6.5 (CS–L), CS + 200 mg/kg sulfate-S + 2308 mg/kg CaCO₃ (CS–SL), HN control (HN), and HN + 200 mg/kg sulfate-S (HN–S). The above-mentioned soil amendments (6.03 g Na₂SO₄·10H₂O and/or 6.92 g CaCO₃) and basal fertilizers (0.84 g CO(NH₂)₂, 0.15 g NH₄H₂PO₄, and 1 g KCl) were mixed with 3 kg of the corresponding soil (oven dried at 40 °C, <2 mm). The mixed soils were placed in watertight pots (inner diameter: 16.5–20.5 cm; height: 22 cm) and flooded with deionized water (day 0). Rice plants were germinated from seeds and grown in nutrient solution (Cock et al., 1976) for 28 days following previously described protocols (Fang et al., 2023). Two seedlings were transplanted into the flooded soil in each pot on day 31. All pots were kept flooded from day 0 until the start of mid-season drying (MSD, soil dried by evapotranspiration) on day 60 (Fig. S1). After MSD (day 60–73), all pots were maintained in an intermittent flooding regime with a seven-day period for each wetting-drying cycle. Pots were weighed every two to three days, which allowed for (evapo)transpiration rate calculation (see Supplementary Materials), and irrigated with a specific amount of deionized water according to the latest evapotranspiration rates to ensure either soil flooding or drying at the next irrigation (Fig. S1). On day 157, rice plants were harvested.

2.2. Soil matric potential

Soil matric potentials (Ψ_m ; Fig. S2) were monitored in two control pots of each soil from day 48 (before MSD) to day 157 (harvest) using miniaturized tensiometers (0.5 cm² ceramic tip surface area, 5 mm diameter, 10 cm shaft length; TEROS31, METEER). Tensiometers were refilled on day 48, 77, 112, 126, and 154 (Fig. S2) before being gently inserted into the flooded soil at 10 cm below soil surface. Readings of Ψ_m were automatically recorded every 5 min by a data logger (ZL6, METEER). Ψ_m of pots without tensiometer deployment and Ψ_m below -80 kPa were calculated from the polynomial fits between Ψ_m (>-80 kPa) and the measured pot weights (see Supplementary Materials; Fig. S3).

2.3. Soil and porewater chemical analyses

Properties of the two soils used in this study are shown in Table S1. Soil redox potential measurements (E_h = oxidation-reduction potential (ORP) + 209 mV; Pt-Ag/AgCl electrodes, Mettler Toledo; 5–8 cm below soil surface) and porewater sampling (Rhizosphere Research Products; 0.6 μ m pore size, 10 cm length) were carried out throughout the experiment under both flooded and drained (measured/sampled 3–4 h after reflooding) soil conditions. One mL of the collected porewater was used immediately for pH measurements with a glass electrode (Mettler Toledo). Four types of porewater samples were immediately preserved (3–5 mL per sample except for sample type (4) where 2 mL were collected): (1) acidified with 50 μ L 30% HCl (Suprapur, Merck) and stored at 4 °C for determining total elemental composition, dissolved organic carbon (DOC), and As species including arsenite, arsenate, DMA and monomethylarsenate (MMA) (note: Due to sample acidification and analysis at acidic pH (see Supplementary Materials), di- and monomethylated thioarsenates would be codetermined with their respective oxyarsenates DMA and MMA (Dai et al., 2021). Thus, DMA and MMA determined from HCl-acidified porewater samples is actually the pooled dimethylated As and monomethylated As species, respectively); (2) stabilized by 300 μ L of 0.1 M Na₂EDTA and stored at 4 °C for determining sulfate concentration; (3) fixed by 250 μ L of 20% Zn acetate and stored at -20 °C for determining sulfide concentration (Burton et al., 2011); (4) (only for samples from day 126 and 140) stabilized by 140 μ L of 57 mM N,N'-Di(2-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid monohydrochloride (HBED; abcr Gute Chemie) solution dissolved in pure ethanol (EMSURE, Merck), flash-frozen by liquid N₂, and stored at -20 °C for determining As speciation including separation of thioarsenates from oxyarsenic species (Knobloch et al., 2024).

Collection days of each type of porewater and the analytical methods for determining total elemental composition, DOC, and As speciation in the HCl-acidified porewater including arsenite, arsenate, pooled dimethylated As, and pooled monomethylated As are described in the Supplementary Materials. Sulfate was analyzed with ion chromatography (940 Professional IC Vario, Metrohm) using a Metrosep A Supp 5 - column (250/4.0, Metrohm; mobile phase: 3.2 mM Na₂CO₃ + 1 mM NaHCO₃, 0.7 mL/min). Sulfide (including sulfide-S in polysulfides (ThomasArrigo et al., 2016)) in freshly thawed Zn-acetate fixed porewater was measured using a modified methylene blue method (Cline, 1969) (λ = 670 nm; Cary 60 UV-Vis, Agilent; details in Supplementary Materials). Arsenic speciation in the thawed HBED-stabilized porewater was determined immediately by IC-ICP-MS (IC: Dionex ICS-3000; ICP-MS: Agilent 8900) using an AG/AS16 IonPac column (Dionex; 2.5–100 mmol/L of NaOH with gradient elution, a flow rate of 1.2 mL/min, and 50 μ L injection volume) (Knobloch et al., 2024).

About 6 g moist soil was collected from scattered spots in each densely rooted pot at rice harvest on day 157 and extracted using 45 mL 0.01 M CaCl₂ (2 h, 23 °C). After centrifugation (3300 g) and filtration (0.45 μ m, nylon filter), the extracted elemental concentrations were measured with inductively coupled plasma mass spectrometry (ICP-MS, Agilent 8800). Based on the 0.01 M CaCl₂ extractable Cd ($Cd_{soil,CaCl_2}$) and

Mn ($Mn_{soil,CaCl_2}$) in moist soils, values for adjusted extractable Cd ($Cd_{soil,CaCl_2}^{adjusted}$), which accounts for the competition by Mn to rice Cd uptake, were calculated using the molar fraction of extractable Cd with respect to the sum of extractable Mn and Cd (Fang et al., 2021):

$$Cd_{soil,CaCl_2}^{adjusted} = Cd_{soil,CaCl_2} \cdot C_{soil,CaCl_2}^{mol/kg} / (C_{soil,CaCl_2}^{mol/kg} + Mn_{soil,CaCl_2}^{mol/kg}) \quad (1)$$

where mol/kg in the superscript indicates molar concentration to be used in calculation.

2.4. Soil DNA analyses

Following soil sampling for CaCl₂ extraction, another ~1 g moist soil was collected from different spots in each pot and stored under -20 °C for soil DNA extraction. Total DNA in moist soil (0.25–0.4 g) was extracted from each pot using the DNeasy® PowerSoil® Pro Kit (Qiagen) in accordance with the manufacturer's protocol. DNA concentrations were determined using Qubit® 2.0 Fluorometer along with the Qubit™ dsDNA HS quantification kit. Quantitative PCR (qPCR) targeting the 16S rRNA gene of bacteria and archaea was carried out using the 341F and 797R primers following the methodology detailed in a previous study (Otte et al., 2018). The DNA from the triplicates of a treatment was combined for amplification, ensuring equimolar DNA quantity from each replicate. Amplification of microbial 16S rRNA genes was executed using the 515F and 806R primers (Caporaso et al., 2010). The quality and quantity of the purified amplicons were determined using agarose gel electrophoresis and NanoDrop 1000 (Thermo Fisher). Subsequent stages of library preparation steps (Nextera, Illumina) and sequencing were performed by NCCT (NGS Competence Center Tübingen) using an Illumina MiSeq sequencing system. In total 1,826,094 paired-end reads with length 250 bp were obtained for six samples (174,968 to 419,175 read pairs per sample). Quality control, reconstruction of 16S rRNA gene sequences, taxonomic annotation, and functional prediction was performed with nf-core/ampliseq (Straub et al., 2020) (version 2.4.1) of the nf-core collection of workflows (Ewels et al., 2020), executed with Nextflow (Di Tommaso et al., 2017) (version 22.04.5) and singularity (Kurtzer et al., 2017) (version 3.8.7), as detailed in the Supplementary Materials. 10,939 amplicon sequencing variants (ASVs) with between 93,626 and 235,797 reads (53% and 62%) per sample were obtained. The raw sequencing data has been deposited at the Sequence Read Archive (SRA) under BioProject accession number PRJNA1026071 (<http://www.ncbi.nlm.nih.gov/bioproject/PRJNA1026071>).

2.5. Plant analyses

At harvest on day 157, rice panicles and the entire roots (after extensive manual washing using deionized water) were collected from each pot. After oven-drying at 40 °C for two weeks, dehusking, and milling (4 min at 24 Hz; MM 200, Retsch), the flour of unpolished rice grains (i.e. brown rice) were digested for determining total elemental composition and enzymatically extracted (Colina Blanco et al., 2021) for As speciation including thioarsenates (details in Supplementary Materials). Root iron-plaque on freshly harvested roots (duplicates of 1 g fresh roots) was extracted with a ascorbate-citrate-acetate (ACA) mixture (Gao et al., 2007) following the previously described protocol (Fang et al., 2023). Roots after ACA extraction were oven-dried, weighed (thus root water content was calculated), milled, and digested using the same microwave digestion program as for rice digestion. The dry weight of all roots from each pot was calculated from root fresh weight (after removing the basal node) and root water content. The enzymatic extraction efficiency was tested using certified rice flour (ERM-BC211, Institute for Reference Materials and Measurements of the European Commission's Joint Research Centre) as the reference material. Another rice flour reference (NIST 1568b, National Institute of Standards & Technology of USA) was used for the digestion of milled

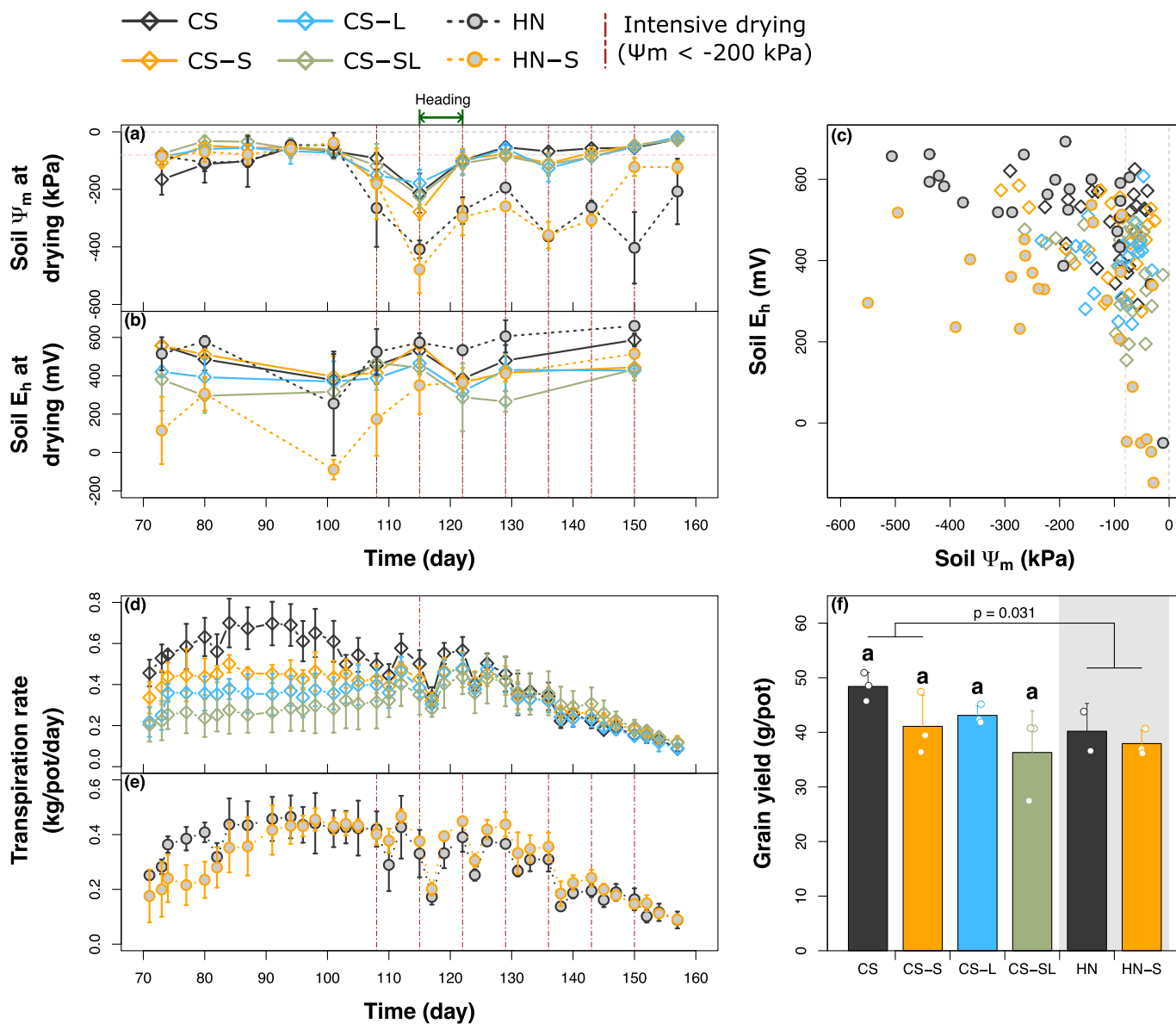


Fig. 1. Soil matric potential (Ψ_m), soil E_h , and plant transpiration rate. Ψ_m and E_h shown in panel (a) and (b) were measured at soil drying periods during intermittent flooding. All Ψ_m and E_h data including during flooded periods can be found in Fig. S2 and Fig. S4a. Bar plots and error bars in panel (f) represent average and standard deviation of grain yield of the six treatments. Yields of individual pots are also shown as open circles within each treatment. Note that one of the HN control pot has one plant showing abnormal growth with no grain formed (Fig. S6), thus this pot was not included for grain yield calculation. Differences ($p < 0.05$) among CS, CS-S, CS-L, and CS-SL treatments according to Tukey’s HSD test are indicated by different letters. The p -value for the comparison between HN and HN-S is 0.53. P -value of the one-sided t -test between the unlimited CS soil and HN soil is also shown.

brown rice. A reference material of branches and leaves of bush (NCS DC 73349, National Analysis Center for Iron & Steel of China) was used for the digestion of roots.

2.6. Statistical analyses

Statistical differences for each selected grain parameter among the four treatments using CS soil (CS, CS-S, CS-L, and CS-SL) and between the two treatments using HN soil (HN and HN-S) were assessed separately by Tukey’s HSD (honestly significant difference) test after one-way ANOVA. All statistical tests and calculation of Pearson correlation coefficient were conducted using R (version 3.6.2) in R studio (version 1.4.1106).

3. Results

3.1. Soil water and plant growth

The soil matric potential (Ψ_m) right before reflooding in each drying period (the driest condition in each cycle) is shown in Fig. 1a. From day 73 to day 101, both soils were dried to similar Ψ_m between -10 and -150 kPa except one CS control pot on day 73 (-227 kPa) to ensure adequate water availability to rice plants. From day 108 to day 150, pots containing the HN soil were allowed to dry further to below -200 kPa. The CS soil-containing pots were still dried to around -100 kPa during day 108–150 except on day 115 where Ψ_m also reached -200 kPa.

Complementary to the Ψ_m measurement which showed water availability to plants at each soil drying, the corresponding soil E_h (Fig. 1b) reflected how well soils were aerated. In drying periods starting

after MSD, the E_h values in the CS soil were 155–625 mV. In comparison, the E_h values in some of the HN soil-filled pots were still below 0 mV on day 73, 101, and 108 where Ψ_m values (−77 to −11 kPa) were even lower than some of the corresponding CS soil (up to −32 kPa) (Fig. 1a–c). To enhance soil aeration at the later drying periods, HN soil was allowed to dry to lower pot weight (Fig. S3c) and Ψ_m (−550 to −87 kPa, Fig. 1a). Sulfate amendment consistently decreased soil E_h in the HN soil after MSD even in drying periods where it showed comparable or lower Ψ_m (Fig. 1b,c and Fig. S4a). Sulfate amendment effect on E_h in CS soil was less pronounced.

As a parameter reflecting plant growth and its response to low soil matric potential, plant transpiration rate since MSD is shown in Fig. 1d and e. A sudden decrease and a quick (partial) recovery of transpiration rate was observed after each intensive soil drying ($\Psi_m < -200$ kPa in general, Fig. 1a) during day 108–150 in the HN soil, while this effect was less evident in the CS soil. During day 129–157, transpiration rates gradually decreased from 0.37 to 0.45 kg/pot/day to 0.09–0.12 kg/pot/day for all treatments. Both sulfate and limestone amendment temporarily decreased plant transpiration rates during the vegetative growth phase before day 103. During the reproductive phase (day 108–157), transpiration rates among treatments were generally similar. When harvested, all pots showed relatively high seed setting rates (82–93%, Fig. S5) and no symptoms of straighthead disease were observed (Fig. S6). Neither sulfate nor limestone amendment showed a clear effect on yield in CS soil ($p = 0.07$ – 0.96) or HN soil ($p = 0.53$), although the average yield from the amended CS soil is up to 25% lower than from the control CS soil (Fig. 1f). When the two soils were compared, the unlimed CS soil showed higher yield than the HN soil (one-sided t -test: $p = 0.031$; Fig. 1f).

3.2. Grain As and grain Cd

In all grain samples, iAs including arsenite and arsenate (0.25–0.66 mg/kg, Fig. 2a) represented the majority (53–94%) of grain As species followed by DMA (0.02–0.21 mg/kg, 5–41%). Only trace amounts of DMMTA (2–16 $\mu\text{g}/\text{kg}$, 0.4–3%), MMA (0–9 $\mu\text{g}/\text{kg}$, 0–2%), and trimethylarsine oxide (TMAO; 0–3 $\mu\text{g}/\text{kg}$, 0–0.6%) were detected. Similar to our previous report under intermittent flooding (Fang et al., 2023), soil sulfate amendment decreased grain iAs concentrations by 47% ($p = 0.002$) in the unlimed CS soil and by 29% ($p = 0.033$) in HN soil. Liming also tended to decrease grain iAs concentration (by 22%, $p = 0.086$) in the CS soil without sulfate amendment. When combining both amendments, it decreased grain iAs by 43% ($p = 0.003$) compared to the unamended CS soil. Sulfate amendment also tended to increase DMA concentration in grains for both soils (unlimed CS: by 46%, $p = 0.065$; HN: by 135%, $p = 0.095$). Remarkably, limestone decreased grain DMA concentrations by 73% ($p = 0.006$) and 81% ($p < 0.001$) in the CS soil with and without sulfate amendment, respectively, leading to 72% less DMA in rice ($p = 0.006$) under combined sulfate and limestone amendment compared to the unamended CS soil.

The limestone amendment alone decreased grain Cd concentration by 36% ($p = 0.005$) in the CS soil (Fig. 2b). Sulfate amendment alone showed no effect on grain Cd ($p = 0.952$) in CS soil but a 44% decrease ($p = 0.010$) in the HN soil. When combining sulfate and limestone amendments, it decreased grain Cd by 68% ($p < 0.001$) compared to the unamended CS soil. No clear amendment effects on Fe, Zn, and P concentrations in brown rice were observed, while sulfate amendment slightly decreased rice Mn concentrations in the HN soil and generally increased rice S concentrations in both soils (Fig. S7).

3.3. Porewater chemistry

Porewater pH throughout the experiment is shown in Fig. 3a–e. Sulfate amendment increased porewater pH of HN soil consistently throughout the experiment with more pronounced effects after MSD. In contrast, the effects of sulfate amendment on porewater pH in CS soil

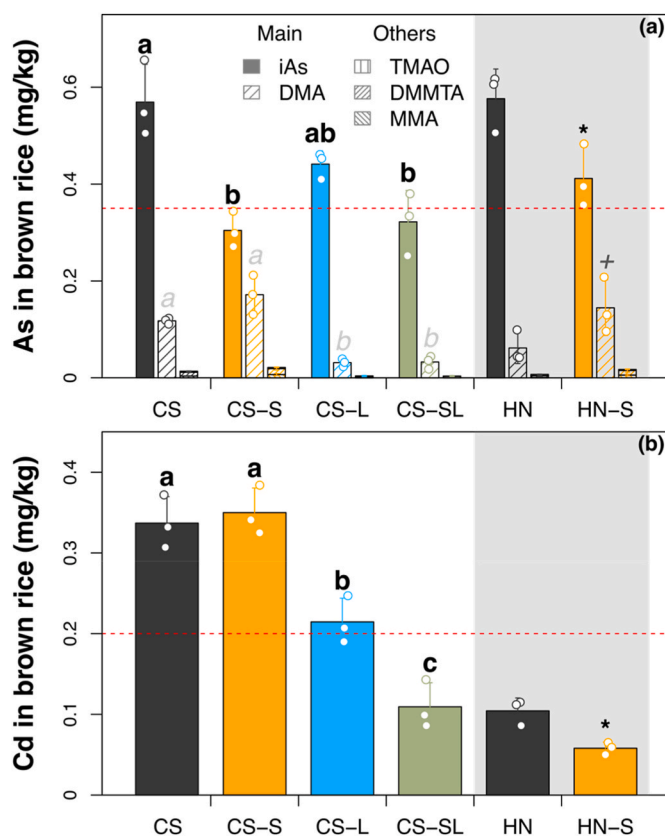


Fig. 2. Concentrations of iAs, different organic As species, and Cd in brown rice. Bar plots and error bars represent average and standard deviations among pot replicates, respectively. Open symbols represent individual data points. (a): Concentrations of As species determined after enzymatic extraction of brown rice. Here, iAs comprised only arsenite and arsenate because inorganic thioarsenates were not detected in grains. (b): Concentration of Cd in brown rice. Red dash line in each panel represents the Chinese regulation limits for iAs or Cd in brown rice, respectively (GB 2762-2022). Differences ($p < 0.05$) among CS, CS-S, CS-L, and CS-SL treatments according to Tukey's HSD test are indicated by different letters. The p -values for the comparison between HN and HN-S are indicated by the label codes below: '+' for $0.05 \leq p < 0.1$; '*' for $0.01 \leq p < 0.05$. Relevant exact p -values are shown in Results and Discussion. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

were weak and inconsistent. Liming clearly increased porewater pH especially during soil drying.

Porewater Fe concentrations throughout the experiment reflected the soil redox conditions well (Fig. 3b–f). After MSD, porewater Fe concentrations were generally < 2.5 mg/L in the drying phase (with a few exceptions) but increased to 5.7–131 mg/L in the flooded phase. Sulfate amendment tended to increase Fe mobilization shortly after each flooding but decrease it after prolonged flooding (days 26–56). In contrast, liming initially decreased porewater Fe after each flooding while having no effects after prolonged flooding (days 52–56), leading to a pronounced liming-induced decrease of Fe mobilization during the intermittent flooding period.

Porewater S and sulfate concentrations gradually decreased to < 10 mg/L and < 2 mg/L, respectively, during soil flooding (days 0–56) in the following order (from earliest to latest): CS $<$ CS-L and HN $<$ CS-S $<$ CS-SL and HN-S treatment (Figs. S4b and c). Soil S was remobilized as sulfate to porewater after MSD. After MSD (days 73–157), porewater S concentrations gradually decreased in the CS soil while they fluctuated in the HN soil. Porewater sulfide quickly increased following each (re) flooding and then stabilized at lower levels for all treatments (Fig. 3c–g). Much higher sulfide concentrations right after MSD were detected in the

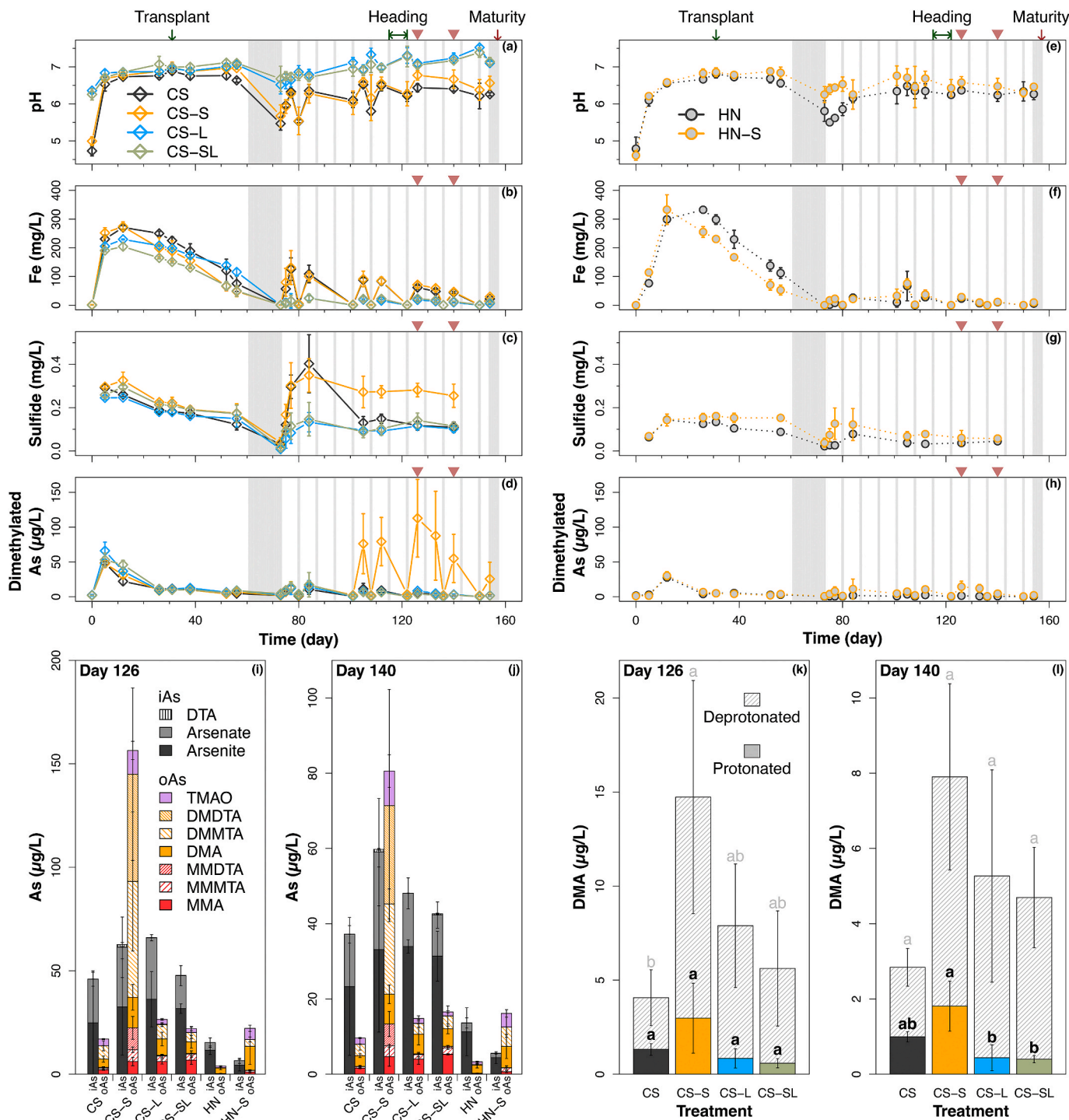


Fig. 3. Porewater pH, Fe, sulfide, and As speciation. Error bars represent standard deviation among treatment triplicates. Shaded background in panels (a)–(h) indicate soil drying. In addition to the thioarsenates-insensitive As speciation throughout the experiment, thioarsenates were determined on days 126 and 140 (marked with red triangle on panels (a)–(h)). Monothioarsenate, trithioarsenate, and monomethyltrithioarsenate in panels (i) and (j) were not shown because their concentrations were close to detection limits (30–60 ng/L As). In panels (k) and (l), concentrations of protonated and deprotonated DMA were calculated using DMA concentrations in HBED-stabilized porewater samples, porewater pH, and the pK_a of DMA at 6.14. Different letters among CS, CS-S, CS-L, and CS-SL indicate $p < 0.05$ according to Tukey’s HSD test for DMA (gray letters) and protonated DMA (black letters), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

unlimed CS soil (0.26–0.52 mg/L) than in the limed CS soil (0.09–0.23 mg/L) and HN soil (0.06–0.20 mg/L). During days 105–140, the plateau concentrations of porewater sulfide were highest in the CS-S treatment (0.25–0.28 mg/L) followed by the CS control, CS-L, and CS-SL (0.09–0.15 mg/L), HN-S (0.06–0.08 mg/L), and the HN control

(0.03–0.04 mg/L).

Temporal variations of As generally followed the trend of Fe in porewater (Fig. 3b–d,f,h and Figs. S4d–g). In the HCl-acidified porewater samples, after a transient peak of arsenate, monomethylated As, and dimethylated As after the initial soil flooding, arsenite (78–91%)

dominated porewater As in the CS soil until day 56 (Fig. 3d and Figs. S4d–g). In the HN soil, in contrast, arsenate (81–95%) was always the dominant porewater As species during days 5–56 (Fig. 3h and Figs. S4d–g). In the flooded periods after MSD, arsenite and arsenate represented the majority of porewater As except for the CS-S and HN-S treatments in which the methylated As species contributed about 50% of porewater As in flooded periods during days 105–154 (Fig. 3d–h and Fig. S4g). Analysis of HBED-preserved porewater samples on days 126 and 140 revealed a substantial contribution of thiolated As species to both monomethylated (9–84%) and dimethylated As (17–90%) except for the control HN soil (Fig. 3i,j and Fig. S9).

Sulfate amendment substantially increased monomethylated and dimethylated As by 2.1–13 and 5.9–22 folds in the porewater of unlimed CS and by 2.9–433 and 1.2–9.9 folds in the HN soil, respectively, in the flooded periods during days 105–154. In the HBED-preserved samples, we found that sulfate amendment to the CS soil not only substantially increased concentrations of monothiolated As species (MMMTA: from 0.11 to 1.3 to 2.6–7.3 µg/L; DMMTA: from 2.0 to 8.5 to 20–91 µg/L) in porewater, it also produced dithiolated As species (MMDTA: 2.9–15 µg/L; DMDTA: 2.4–83 µg/L) which were hardly detected in the porewater of other treatments (MMDTA: 0–0.72 µg/L; DMDTA: 0–0.55 µg/L). Liming to the sulfate-amended CS soil greatly decreased methylated and thiolated As species in the porewater, leading to a similar profile of As species in the porewater between CS-SL and CS-L treatments (Fig. 3d–i,j and Fig. S4g). Without sulfate amendment, liming alone slightly increased porewater monomethylated and dimethylated As in CS soil. Irrespective of the sulfate amendment, the HN soil showed similar or less arsenate and much less arsenite, methylated As species, and thiolated As species in porewater compared to the unlimed CS soil after MSD (Fig. 3d, h–j and Figs. S4e–g). This may be due to the high soil Mn concentration in the HN soil which may have slowed down the reductive solubilization of As as arsenite by poisoning the E_h and/or re-oxidizing released arsenite followed by re-adsorption of arsenate to solid phases. Due to the much lower porewater concentration of arsenite, much less methylated and thiolated As species can be formed using arsenite as the source material.

Cd concentrations in porewater were very low except on day 0 and during days 56–80 (Fig. S4h). A decrease in porewater silicon (Si) during the intermittent flooding period (day 73–157) indicating an immobilization of Si was observed in both soils (Fig. S8a), consistent with our previous report (Fang et al., 2021; Fang et al., 2023). Porewater concentrations of Mn, DOC, N, P, K, Mg, Zn, Na, Ca, Cu, and Al are also shown in Fig. S8.

3.4. Root iron-plaque and roots

After sulfate amendment, 28–38% less Fe ($p = 0.024–0.085$), 24–34% less As ($p = 0.011–0.075$), and 38–53% less Cd ($p = 0.097–0.366$) per unit root dry mass were extracted from the root iron-plaque in both CS and HN soils (Fig. S7). Liming of the CS soil showed no effects on Fe and As content but tended to increase Cd content ($p = 0.056–0.225$) in iron-plaque. Sulfate amendment also decreased root Fe and As concentrations in the unlimed CS soil, while the effects were weaker in the limed CS soil. For the HN soil, sulfate amendment had little effects on root Fe ($p = 0.750$) and As ($p = 0.279$) concentrations. Comparing the two soils, higher Fe and As concentrations in iron-plaque and roots were found in the HN soil than the CS soil even though the latter showed higher porewater Fe (without liming) and As after MSD. Combining all 18 samples, a positive correlation ($r = 0.68$, $p = 0.002$) between grain iAs and As in root iron-plaque was found (Fig. S10).

3.5. Moist soil extractable Cd and Mn

Extractable Cd, Mn, and the adjusted extractable Cd concentrations in moist soils collected at harvest are shown in Fig. 4a–c. Sulfate amendment decreased extractable Cd in the unlimed CS soil by 23% ($p = 0.005$) and tended to decrease it in the limed CS soil ($p = 0.637$) and

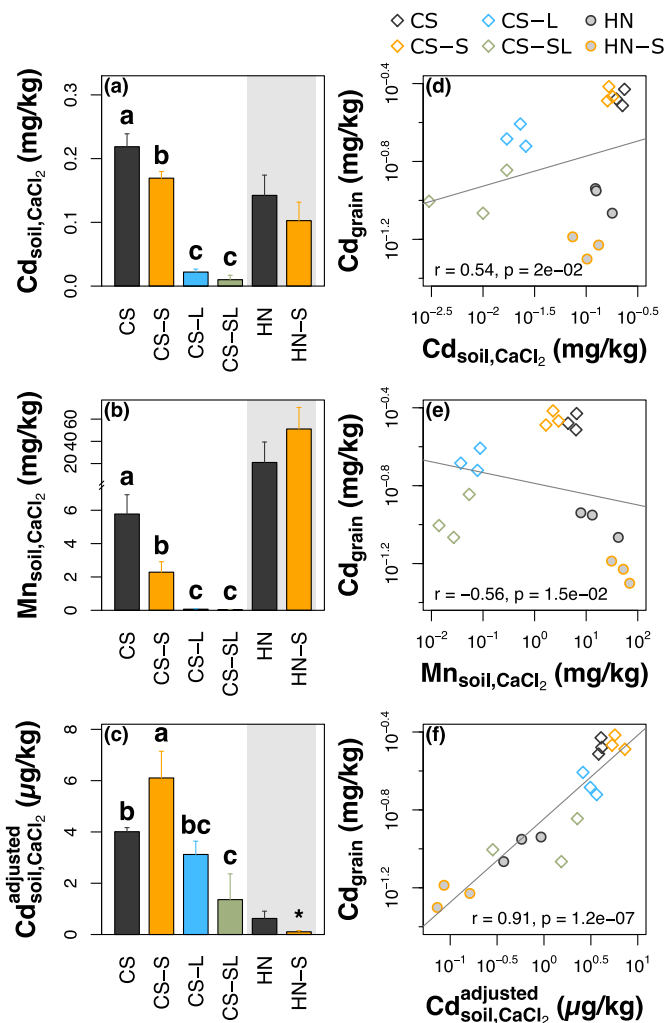


Fig. 4. Extractable Cd and Mn in moist soils at harvest using 0.01 M CaCl_2 . Barplots and error bars represent average and standard deviations among pot triplicates, respectively. Differences between treatments ($p < 0.05$) according to Tukey's HSD test are indicated by different letters for CS soil (CS, CS-S, CS-L, and CS-SL) and by the label codes below for HN soil (HN and HN-S: '**' for $p < 0.05$). Relevant exact p-values are given in Results and Discussion. Pearson correlation coefficients and the corresponding p-values are shown for panels (d)–(f).

HN soil ($p = 0.185$). Liming substantially decreased soil extractable Cd by 90% ($p < 0.001$) and 94% ($p < 0.001$) in the control and sulfate-amended CS soil, respectively. Sulfate amendment also decreased soil extractable Mn in the unlimed CS soil by 60% ($p < 0.001$) but tended to increase it in HN soil ($p = 0.123$). Liming decreased extractable Mn in the CS soil drastically from 1.7 to 6.5 mg/kg to < 0.1 mg/kg ($p < 0.001$) irrespective of sulfate amendment. Comparing soils, extractable Mn was much higher in HN soil (7.9–70 mg/kg) than in unlimed CS soil (1.7–6.5 mg/kg) while their extractable Cd contents were similar. Consequently, the calculated adjusted extractable Cd followed the order $\text{CS-S} > \text{CS} \geq \text{CS-L} \geq \text{CS-SL} \geq \text{HN} > \text{HN-S}$ (Fig. 4c). While both soil extractable Cd ($r = 0.54$, $p = 0.020$; Fig. 4d) and extractable Mn ($r = -0.56$, $p = 0.015$; Fig. 4e) only weakly correlated to grain Cd, the adjusted soil extractable Cd highly and positively correlated ($r = 0.91$, $p < 0.001$; Fig. 4f) with grain Cd.

3.6. 16S rRNA gene abundance of potential Fe(III)- and sulfate-reducers in soils

The total copy numbers of 16S rRNA genes among treatments were

similar in the CS soil while they were slightly higher under sulfate amendment in the HN soil (Fig. 5a). Among the major genera (maximum relative abundance among samples >0.1%) of potential Fe(III)-reducers and sulfate-reducers, *Desulfovibrio*, *Desulfosporosinus*, and *Desulfotobacterium* were identified as shared genera, i.e., containing Fe(III)-reducers and sulfate-reducers. Except of these three shared genera, sulfate amendment did not show consistent effects on the relative 16S rRNA gene abundance of potential Fe(III)-reducers in the unlimed and limed CS soil and the HN soil. Liming consistently increased the relative abundance of *Geobacter* in the CS soil from <0.02% to >0.18% irrespective of sulfate amendment (Fig. 5b). Sulfate amendment greatly increased the relative abundance of all major genera of potential sulfate-reducers except for *Haliangium* (Fig. 5c). Liming decreased the relative abundance of *Desulfosporosinus*, *Desulfoprimum*, *Desulfotobacterium*, and *Haliangium* regardless of sulfate amendment. Additionally, liming only decreased the fraction of *Desulfovibrio* without sulfate amendment and had limited influence on *Desulfovibrio* and *Syntrophobacter*. Overall, liming induced a decrease of the combined relative abundance of potential sulfate-reducers (Fig. 5c).

4. Discussion

4.1. Role of soil texture in producing low-As rice via intermittent soil flooding

By monitoring soil matric potentials, soil E_h , and plant transpiration rates throughout the pot experiment, we showed how soil texture can influence the tradeoff between soil aeration and soil water availability to rice plants in the context of cultivating rice with low As accumulation. When both soils were drained to similar Ψ_m values (>−200 kPa) with no sign of plant water stress, the clay loam HN soil showed less complete aeration compared to the sandy loam CS soil. Notably for Ψ_m between −80 and −10 kPa, the CS soil was consistently well aerated to $E_h = 155$ –625 mV with <10.2 mg/L Fe and <29 $\mu\text{g/L}$ As in porewater, while HN soil still showed cases with $E_h < 0$ mV (Fig. 1b and c) with up to 54 mg/L Fe and 47 $\mu\text{g/L}$ As in porewater (Fig. 3b–f, Fig. S4d). The higher porewater As concentrations in the HN soil during drying periods here are particularly noteworthy considering that Fe and As mobilization after soil flooding was already much slower in the HN soil than in the CS soil (Fig. S4d). It implies an even higher risk of rice As uptake in fine-textured soils with faster reductive dissolution of As. When the HN

soil was further aerated to limit soil As mobility, it showed a more rapid decrease of Ψ_m below −200 kPa along with the transpiration-induced soil water loss compared to the CS soil (Figs. S3a and b). This can be explained by the smaller pore sizes and stronger water retention by soil surfaces in the finer-textured HN soil (Blume et al., 2016). In this case, plant stress response to the decreased water availability in the HN soil was evidenced by the larger temporary decrease of transpiration rates than those in the CS soil (Fig. 1d and e). The higher water stress in the HN soil during drying periods may have contributed to its slightly lower rice yield than the unlimed CS soil (Fig. 1f). Therefore, our results suggest that it can be more difficult to manage finer-textured soils under intermittent flooding to ensure soil aeration needed for controlling rice As accumulation and sufficient water availability to rice plants simultaneously.

4.2. Combined effects of sulfate and limestone amendment on porewater chemistry

Suppression of Fe mobilization (Fig. 3b) and sulfate reduction (Fig. 3c and Figs. S4b and c) by soil liming was observed in the porewater of the flooded CS soil, especially in the intermittent flooding phase after MSD. The liming-induced suppression on sulfate reduction was more profound in the sulfate-amended CS soil than in the control CS soil (Fig. 3c). Strong suppression of As methylation (Fig. 3d and Fig. S4g) and thiolation (Fig. 3i and j) by liming was also evidenced in porewater during days 105–154 in sulfate-amended CS soil. This is expected from the observed liming effects on sulfate reduction (Fig. 3c), since sulfate-reducers and reduced S species (elemental S and sulfide) play a key role in As methylation and thiolation, respectively (Wang et al., 2020; Chen et al., 2019). Without sulfate amendment, an increase instead of a consistent decrease of porewater monomethylated As, dimethylated As, and thioarsenates by liming was observed during days 105–154 (Fig. 3d–i,j and Fig. S4g). This can be explained by the low production of monomethylated As, dimethylated As, and thioarsenates in the control CS soil and their likely increased desorption as anions from solid phases under higher pH considering their pK_a values: DMA, 6.14;⁴⁷ MMA, 4.19;⁴⁷ DMMTA, 5.39 (calculated using Chemicalize by Chemaxon); DMDTA, 2.25 (calculated using Chemicalize by Chemaxon).

The decreased Fe mobilization by liming may not be due to a decrease in Fe(III) reduction, since liming showed limited influence on the (relative) abundance of the potential Fe(III)-reducing bacteria in the

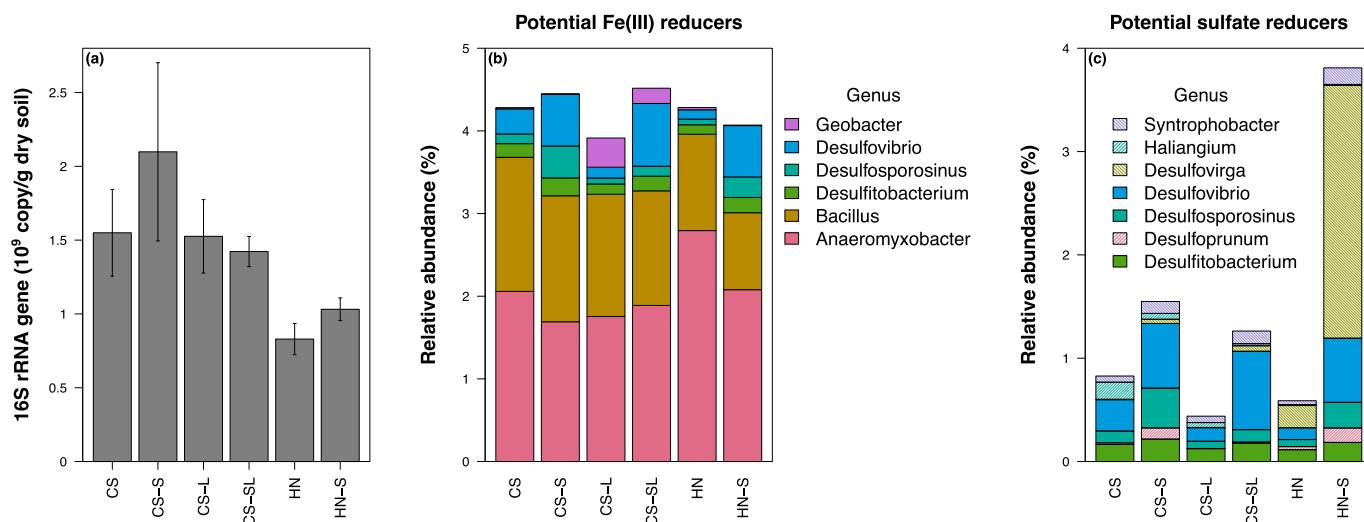


Fig. 5. Total 16S rRNA gene copy numbers (determined by qPCR) and relative abundance of 16S rRNA gene sequences of potential Fe(III)- and sulfate-reducing prokaryotes (determined by amplicon sequencing) in soil. The list of genera containing potential Fe(III)-reducers were compiled according to Weber et al., 2006. Putative sulfate-reducers were predicted to carry dissimilatory sulfite reductase enzyme (EC:1.8.99.5) by PICRUSt2 (Douglas et al., 2020) based on amplicon sequence variants (ASVs). For clarity, only genera with relative abundance higher than 0.1% are shown.

CS soil. Thus, the increased Fe(II) adsorption to soil solid phases and/or precipitation at higher porewater pH (limed soil: 6.6–7.2; unlimed soil: 5.9–6.8) may be responsible for the decreased Fe mobilization under liming after MSD. Suggested by the decreased overall (relative) abundance of the major potential sulfate-reducers under liming (Fig. 5c), the suppression of sulfate reduction, As methylation, and As thiolation in the CS soil porewater by liming may be related to the inhibited growth of sulfate-reducers. However, further studies are needed to elucidate whether and how liming to acidic paddy soils can affect the abundance and activity of Fe(III)-reducers and sulfate-reducers under flooded conditions.

4.3. Protonated DMA in porewater explained grain DMA

In this study, we showed that liming decreased grain DMA by >70% irrespective of sulfate amendment (Fig. 2a). This result differs from a field experiment in which limestone amendment did not affect rice grain DMA (Chen et al., 2018). Such discrepancy may be due to the different soil As methylation potential and water management between the two studies. For the sulfate-amended CS soil during days 105–154, the large decrease of grain DMA by liming was consistent with the decrease of dimethylated As species in pore water (Fig. 3d). On days 126 and 140 where thiolated species within dimethylated As were further differentiated in the HBED-stabilized porewater, liming decreased porewater DMA concentrations by 20–80% in the sulfate-amended CS soils (Fig. 3k, l). However, the 73% decrease of grain DMA induced by liming to the control CS soil was not reflected in a similar decrease of porewater dimethylated As (including oxy- and thio-arsenates) and, specifically, DMA concentrations when comparing CS and CS-L (Figs. 2a and 3d–i,j) unless the difference in porewater pH was considered.

Lower DMA uptake by rice plants at higher pH of nutrient solution has been shown (Li et al., 2009), which was explained by the decreased proportion of the protonated DMA that is taken up faster by rice plants than the deprotonated DMA. In this study, liming clearly increased porewater pH from 5.9–6.8 to 6.6–7.2 during flooded periods after MSD irrespective of sulfate amendment (Fig. 3a). This corresponds to a 42–79% decrease of the protonated DMA fraction (i.e. from 19–43% to 7.4–15% of porewater As) during days 105–154, taking a $pK_a = 6.14$ for DMA (Zhang and Selim, 2008). Combining the quantified DMA concentrations in the HBED-stabilized samples and porewater pH on days 126 and 140, we show that liming decreased the protonated DMA concentrations in the porewater by 46% and 79% in the unamended and sulfate-amended CS soil, respectively (Fig. 3k,l). Therefore, the proportion change of the protonated DMA can not only account for the grain DMA differences between CS and CS-L (Figs. 2a and 3k,l), it may also contribute significantly to the lower grain DMA concentration in CS-SL than in CS-S in addition to their differences in porewater DMA concentrations.

It is also important to consider the contribution of DMMTA in porewater to DMA accumulation in rice grains. This is due to the fact that DMMTA had similar or, in the cases of CS-S and HN-S, even much higher concentrations compared to DMA in porewater, and DMMTA was shown to be taken up by rice plants even more efficiently than DMA (Kerl et al., 2019). Due to the structural similarity between DMMTA and DMA, the higher uptake efficiency of the protonated form than the deprotonated form may also apply to DMMTA. One crucial but missing information for understanding rice uptake of DMMTA is its precise pK_a value. Assuming pK_a of DMMTA (calculated to be 5.39 using Chemicize by Chemaxon) is lower than DMA ($pK_a = 6.14$) due to the substitution of O atom with S atom, the liming-induced pH increase from 5.9–6.8 to 6.6–7.2 can also decrease the proportion of protonated DMMTA and potentially its uptake by rice plants. Last but not least, both DMMTA dethiolation and DMA thiolation can happen in the rice plant even after uptake, further shifting the final relative share of the two species in the grain (Colina Blanco et al., 2023). However, details on DMMTA uptake by rice plants including the responsible transporters and

abiotic or enzymatically catalyzed transformations inside the plants are still largely unknown and further studies are needed.

4.3.1. Arsenic in root iron-plaque reflected grain iAs better than porewater iAs

The effects of sulfate and limestone amendment on porewater chemistry cannot explain the differences of grain iAs among samples. The lower Fe and iAs mobilization of CS-SL compared to CS-S does not match the similar grain iAs contents between CS-SL and CS-S. The relation between porewater iAs and grain iAs was also unclear when comparing CS-S and CS-L to CS. The obvious decoupling between porewater iAs and grain iAs was evidenced when comparing the two soils. When flooded after MSD, the HN soil showed lower arsenate and much lower arsenite concentrations in porewater (Figs. S4e and f), but similar or even higher grain iAs concentrations than the unlimed CS soil, irrespective of sulfate amendment (Fig. 2a).

Instead, the differences of grain iAs among treatments or between the two soils can be related to As in root iron-plaque which was previously shown to be mainly iAs (Yamaguchi et al., 2014; Seyfferth et al., 2010). The positive correlation between grain iAs and As in root iron-plaque (Fig. S10) agreed with the recent findings (Fang et al., 2023; Liu et al., 2021b) which supported the hypothesis that As in root iron-plaque may be a local iAs source for rice uptake (Fang et al., 2023). Thus, the similar or higher grain iAs of the HN soil can be explained by its similar or higher As content in root iron-plaque compared to the CS soil (Fig. S10). The decreased grain iAs by sulfate amendment can also be explained by the decreased As loading on root surfaces in both soils. The discrepancy between As in root iron-plaque and iAs in porewater can be ascribed to the fact that the amount of root iron-plaque also depends on other factors in addition to porewater iAs concentration. Sulfide has been suggested to account for the decreased iron-plaque on rice roots after soil sulfate amendment (Fang et al., 2023) by restricting root radial oxygen loss (Armstrong and Armstrong, 2005) and/or dissolving ferrihydrite (ThomasArrigo et al., 2020) on roots. The lower porewater sulfide concentrations in the HN soil than in the CS soil (Fig. 3c–g) likely explained their similar As contents in root iron-plaque despite the much lower porewater iAs in the HN soil.

4.4. The adjusted soil extractable Cd explained grain Cd accumulation

The different grain Cd concentrations among samples can be well explained by the adjusted soil extractable Cd (Fig. 4f), which takes into account the competition between soil extractable Mn and Cd for plant uptake and grain accumulation (Fang et al., 2021). Both dissolved Mn^{2+} and dissolved Cd^{2+} enter rice plants mainly through the same transporter OsNramp5 (Sasaki et al., 2012). Mn has also been shown to compete with Cd uptake or accumulation in rice grain in both lab and field studies (Fang et al., 2021; Yang et al., 2014). Different from the previous report where soil extractable Mn only showed a small contribution (accounting for <20% of the R^2) in predicting grain Cd from soil extractable Cd and Mn, (Fang et al., 2021; Fang et al., 2022) we show that the adjustment of soil extractable Cd by soil extractable Mn greatly improved its correlation to grain Cd (from $r = 0.54$ to $r = 0.91$). This is likely due to the larger variation in soil extractable Mn (0.014–70 mg/kg, 5000-fold variation) than soil extractable Cd (0.003–0.24 mg/kg, 80-fold variation) among samples in the present study.

The treatment effects on grain Cd can also be well explained by the effects on the adjusted soil extractable Cd. Sulfate amendment decreased soil extractable Cd but also soil extractable Mn in the unlimed CS soil, leading to even higher adjusted soil extractable Cd and slightly higher rice Cd in CS-S than in the CS control. The lower grain Cd from the HN soil than from the unlimed CS soil can be explained by the much higher soil extractable Mn and, thus, the much lower adjusted soil extractable Cd in the HN soil, although soil extractable Cd concentrations were similar in both soils. Therefore, we show that the adjusted soil extractable Cd, which takes soil extractable Mn into account, is more relevant

to estimating grain Cd than soil extractable Cd alone and should be considered when assessing soil Cd availability to rice plants.

5. Conclusions

In this study, we showed that soil texture can play an important role when optimizing water regime for safe rice production in the trade-off between sufficient yield, low grain iAs, and low grain Cd. Specifically, rice cultivated in the finer-textured HN soil (clay loam; Fig. 1) was more prone to water stress during soil aeration intended to decrease As in rice compared to the sandy loam CS soil. Thus, the water management strategy, as a key factor controlling yield (via water stress (Carrizo et al., 2017) or DMA-induced straighthead disease (Fang et al., 2023; Wells and Gilmour, 1977)), grain iAs, and grain Cd, should be optimized to the local soil conditions and coupled with soil- and/or plant-targeted strategies when necessary to secure the quality and quantity of rice production.

For the sandy loam CS soil, our results showed that combining sulfate and limestone amendment under intermittent flooding can effectively and simultaneously decrease concentrations of iAs, DMA, and Cd in rice grain with limited impact on yield. The beneficial combined effects among sulfate amendment, liming, and intermittent soil flooding can be summarized as below. The soil sulfate amendment decreased iAs in rice, while the risk of increasing rice DMA was avoided by intermittent soil flooding (Fang et al., 2023) and liming. This is likely because liming substantially decreased the protonated DMA (which is taken up more quickly by rice plants than deprotonated DMA (Li et al., 2009)) in porewater and, thus, DMA accumulation in rice. Intermittent flooding boosted sulfate amendment effects in decreasing rice iAs but can lead to higher Cd in rice compared to continuous soil flooding (Fang et al., 2023), where the Cd risk was also avoided by liming. This is because liming decreased soil Cd availability (as the adjusted soil extractable Cd) to rice considering its effects on both soil extractable Cd and Mn. Thus, our data suggest that combining soil sulfate amendment, liming, and intermittent flooding can support the safe rice production from coarse-textured soils when both As and Cd are of concern, which should be tested in field trials in the future.

CRedit authorship contribution statement

Xu Fang: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Andrea E. Colina Blanco:** Writing – review & editing, Validation, Methodology, Investigation. **Iso Christl:** Writing – review & editing, Supervision, Conceptualization. **Maureen Le Bars:** Writing – review & editing, Validation, Methodology, Investigation. **Daniel Straub:** Writing – review & editing, Validation, Software, Methodology. **Sara Kleindienst:** Writing – review & editing, Methodology. **Britta Planer-Friedrich:** Writing – review & editing, Validation. **Fang-Jie Zhao:** Writing – review & editing, Project administration, Funding acquisition. **Andreas Kappler:** Writing – review & editing. **Ruben Kretzschmar:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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.

Data availability

Data is available at: <https://doi.org/10.3929/ethz-b-000664071>.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2024.123786>.

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