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Alternate wetting and drying limits arsenic in porewater and rice grain under severe future climate conditions

*Aria H. Duncan[1,2], Natalia Armenta[1], Frida Garcia-Ledezma[1], Celine A. Heck[1], Sylvia Hafner[3], Britta Planer-Friedrich[3,†], and Scott Fendorf [1,]**

[1]Earth System Science Department, Stanford Doerr School of Sustainability, Stanford University, CA 94305, United States

[2]Applied Microbial Ecology Department, Helmholtz Centre for Environmental Research, 04318, Leipzig, Germany

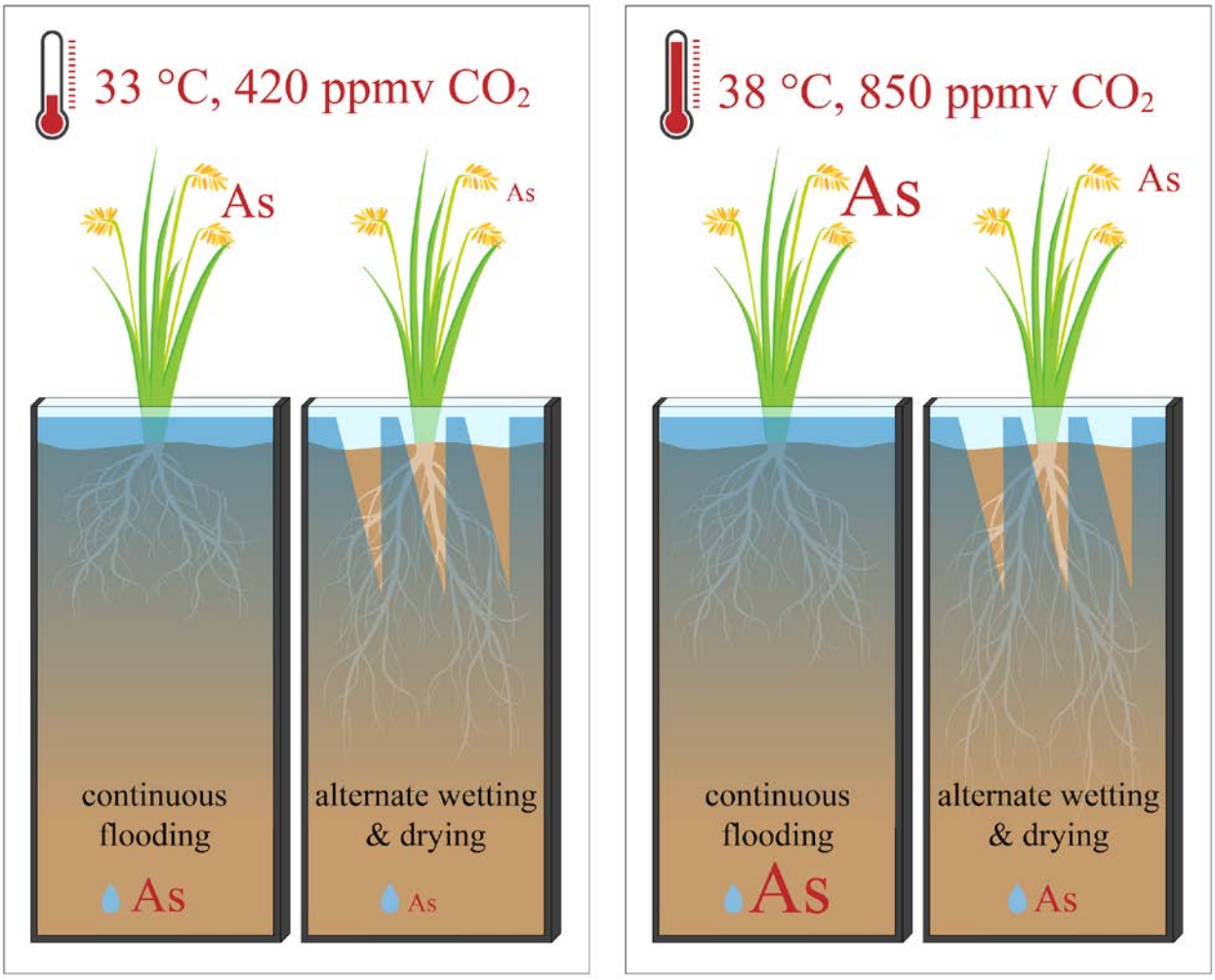
[3]Environmental Geochemistry, Bayreuth Center for Ecology and Environmental Research, University of Bayreuth, 95440, Bayreuth, Germany

*Corresponding author email: fendorf@stanford.edu

†Deceased January 2024

ABSTRACT: Climate change coupled with widespread soil arsenic (As) contamination are expected to decrease rice yields and increase grain As, threatening food security. One promising mitigation strategy is alternate wetting and drying (AWD) irrigation. However, AWD has not previously been tested under potential future climate conditions. Using rhizoboxes to visualize the

rhizosphere, we evaluated the efficacy of AWD for limiting porewater and grain As under both current (daily high of 33 ºC and 420 ppmv CO_2) and severe warming conditions (daily high of 38 ºC, 850 ppmv CO_2). Compared to continuous flooding, AWD decreased cumulative As exposure 10 cm below the surface by 8.2x under the 33 ºC climate and by 15.9x under the 38 ºC climate. Grain total As concentrations decreased by 1.5x with AWD under the 33 ºC climate and by 1.3x under the 38 ºC climate. Porewater cadmium (Cd) concentrations often increased following drainage but never exceeded 1 µg L^{-1}, and grain Cd concentrations were 14.7x to 119.7x lower than grain As concentrations. Both AWD and the 38 ºC and 850 ppmv CO_2 climate condition enhanced root growth. Our findings indicate that AWD may still be an effective As mitigation strategy under severe future climate conditions.



KEYWORDS: intermittent irrigation, arsenic, rice, climate change, cadmium, irrigation management

SYNOPSIS: Arsenic poisoning from consuming contaminated rice is a serious public health issue. This study reports that irrigation management can decrease the amount of arsenic in rice grain even under a severe warming scenario.

INTRODUCTION

Rice is a crucial staple food source. More than half of the global population consumes rice daily,[1] and the demand for rice is increasing per capita and with population growth.[2–4] Hence, sustainable intensification of rice production is needed to minimize environmental degradation and provide sufficient food, particularly in the face of a changing climate. However, many rice-producing regions have soil and irrigation water contaminated with geogenic and/or anthropogenic arsenic (As) and cadmium (Cd),[5,6] which are both Group 1 human carcinogens.[7] Rice, in fact, is recognized as an important source of dietary exposure to both As and Cd.[8–13]

Rice is particularly vulnerable to As contamination because flooded paddy soils become anaerobic, encouraging microbially-mediated carbon oxidation coupled with the reductive dissolution of As(V)-bearing ferric iron (Fe(III)) (hydr)oxides.[14–20] Methylated oxyarsenates, of which dimethylarsinic acid (DMA(V)) is generally the most abundant, are also produced in paddy soils and accumulate in rice grains;[21–24] these species are toxic to plants, causing straighthead disease,[25] but they are considered less toxic for humans and are not included in European Union, United States, or Chinese dietary limits.[26–28] Currently, the European Union and Chinese regulatory maximum concentration of inorganic arsenic (often assumed to be only the sum of arsenite and arsenate) in polished rice grain is 200 μg kg^{-1}.[26,27] The United States only regulates inorganic arsenic in infant rice cereals, and the limit is currently 100 μg kg^{-1}.[28]

Thiolated As species, particularly dimethylmonothioarsenate (DMMTA(V)), are an emerging concern as mounting evidence indicates that DMMTA(V) is highly toxic for plants and humans and ubiquitous in rice products.[29–31] Previously, DMMTA(V) was incorrectly measured as the less toxic, unregulated species DMA(V). DMMTA(V) concentrations in rice range from 0 to 21% of total As and should not be overlooked due to its toxicity to plants and humans.[29,30,32]

Unlike As, Cd is less available under reducing conditions because the microbially-mediated reduction of sulfate enables the formation of poorly-soluble Cd-sulfide, and flooding can raise the pH of an acidic soil promoting Cd^{2+} sorption, primarily on or with carbonates.[33] Additionally, the reductive dissolution of manganese (Mn) oxides also allows Mn^{2+} to compete with Cd^{2+} for uptake into roots.[34] These opposite responses to soil redox conditions make the simultaneous management of As and Cd challenging.

The 2021 IPCC SSP5-8.5 projection for the year 2100, which assumes no decrease in global emissions, is 3.3–5.7 ºC of warming with a mean of +4 ºC. Since previous rice studies simulated a future climate with +5 ºC and 850 ppmv CO_2, we used the same severe warming scenario for consistency.[35] Rising temperatures are expected to decrease yields and increase grain As concentrations as a result of increased soil microbial activity.[35–38] However, several promising mitigation strategies have been identified including various soil amendments and alternate wetting and drying (AWD) irrigation (sometimes referred to as intermittent flooding), which involves periodically drying and reflooding paddies to favorably influence soil biogeochemistry. Numerous studies indicate that AWD has the potential to simultaneously decrease As uptake, water use, and methane emissions.[39–66] However, drainage can increase grain Cd and nitrous oxide emissions, and if drying is too severe it can cause yield losses.[42,43,49,52,55,56,58,67–72] It is therefore essential to limit drainage duration and severity and carefully time nitrogen fertilization to avoid pulses of nitrous oxide that offset decreases in methane production. However, it is possible to sustain and even increase rice yields with carefully managed AWD.[39,44,53,55–57,63,64,72–74]

Although many studies have demonstrated the potential of AWD irrigation under current climate conditions, we are not aware of any that test AWD under potential future climate conditions. Here,

we examine how severe temperatures and atmospheric carbon dioxide (CO_2) concentrations alter the effects of AWD on rice paddy biogeochemistry and the consequences for rice grain As and Cd contamination.

MATERIALS AND METHODS

Soil and rice characterization

The rice (*Oryza sativa* L.) varietal M206, sub-species japonica, cultivar Calrose, seeds were produced in 2019 at the California Rice Experiment Station in Biggs, CA, USA (39º27'51.6"N 121º44'2.7"W). Soil was obtained from a rice farm in Woodland, CA, USA (38º50'52.4"N 121º52'3.4"W) in May and December 2021 (fresh soil was used for each experiment). The same varietal (M206) was grown there from 2005 to 2020, but the field was fallowed in 2021 due to drought. Rice straw and roots were incorporated into the soil; however, large intact root clumps were taken out by hand during soil homogenization. Detailed soil characterization is provided in Supporting Information Table S1. Briefly, the soil is a Sacramento clay, taxonomically classified as a very-fine, smectitic, thermic Cumulic Vertic Endoaquolls.[75] The soil pH is 7.6, and the cation exchange capacity is 28.85 ± 0.37 cMol charge kg^{-1} soil with 1.92% organic carbon and 0.21% nitrogen by weight, determined using an elemental analyzer (Thermo FlashSmart Elemental Analyzer, Thermo Fisher Scientific Inc., USA). The soil has 65.60 mg kg^{-1} soil plant-available nitrogen (18.38 mg kg^{-1} soil nitrogen as ammonium and 47.23 mg kg^{-1} soil nitrogen as nitrate, measured using potassium chloride (KCl) extraction (2 g soil to 20 mL of 2 M KCl) and a discrete analyzer (WestCo SmartChem 200 discrete analyzer, Westco Scientific Instruments Corp., USA)). The phosphate concentration is 12.52 mg kg^{-1} soil (measured using sodium bicarbonate ($NaHCO_3$) extraction (2 g soil to 20 mL 0.5 M $NaHCO_3$) and a discrete analyzer). Total soil As = 5.5 mg kg^{-1}, and soil Cd = 0.0097 mg kg^{-1}.

Greenhouse Experiments

Rice was grown in rhizoboxes within climate-controlled 1.2 x 3.6 x 1.8 m polycarbonate chambers within a larger greenhouse (Figure S1B) as described previously.[35] Chamber temperatures were controlled using heaters (PHM-1 1500-Watt Portable Milkhouse Heater, King Electric, USA), fans (HT-908 TurboForce Fan, Honeywell International Inc., USA), and an air conditioning unit (SereneLife SLCAP8 portable AC, SereneLife Home LLC., USA) connected to an automated sensor/controller system (Inkbird ITC-310T-B Digital Temperature Controller, Inkbird Technology, China) programmed with a diurnal temperature cycle with a daily high of 33 ºC for the climate chamber representing today's climate in a rice-producing region and a daily high of 38 ºC for the simulated severe warming climate chamber (the IPCC SSP5-8.5 projection for the year 2100 is 3.3 ºC to 5.7 ºC of warming).[76] The atmospheric CO_2 concentration in the severe climate chamber was continuously elevated to ≈850 ppmv CO_2 by injecting 99.9% industrial grade CO_2 in front of a fan with a gas flow rate automatically adjusted by a regulator and sensor/controller (Titan Atlas9 CO_2 Controller, Titan Controls, USA) with sensors at canopy level. The entire atmosphere of each growth chamber was exchanged ten times per hour.

We conducted growth experiments in two successive years to provide replication. Results from experiment replicate 1 are presented in the main text, as this experiment lasted the full duration of a growing season and provided the grain data. Results from the 2-month replicated experiment, referred to as experiment replicate 2, are provided in the Supporting Information.

Custom rhizoboxes (dimensions: 81.3 cm x 35.6 cm x 2.5 cm inner width) were constructed out of black and transparent 0.60 cm (front and back faces) and 1.27 cm (sides and bottom) thick acrylic (Figure 1A). Support frames cut from 0.64 cm thick 5052 aluminum sheet metal minimized

bulging and leaking by distributing the force of the screws along the edges. Caulking tape was used as a gasket and waterproof Flex Glue was also applied along the seams. A valve and 4 cm thick layer of quartz sand at the bottom of each box was used to emulate slow downward water percolation. Each rhizobox was packed with ≈10 kg of dry soil. Six rhizobox replicates were used each containing a single plant (six biological replicates per experimental condition).

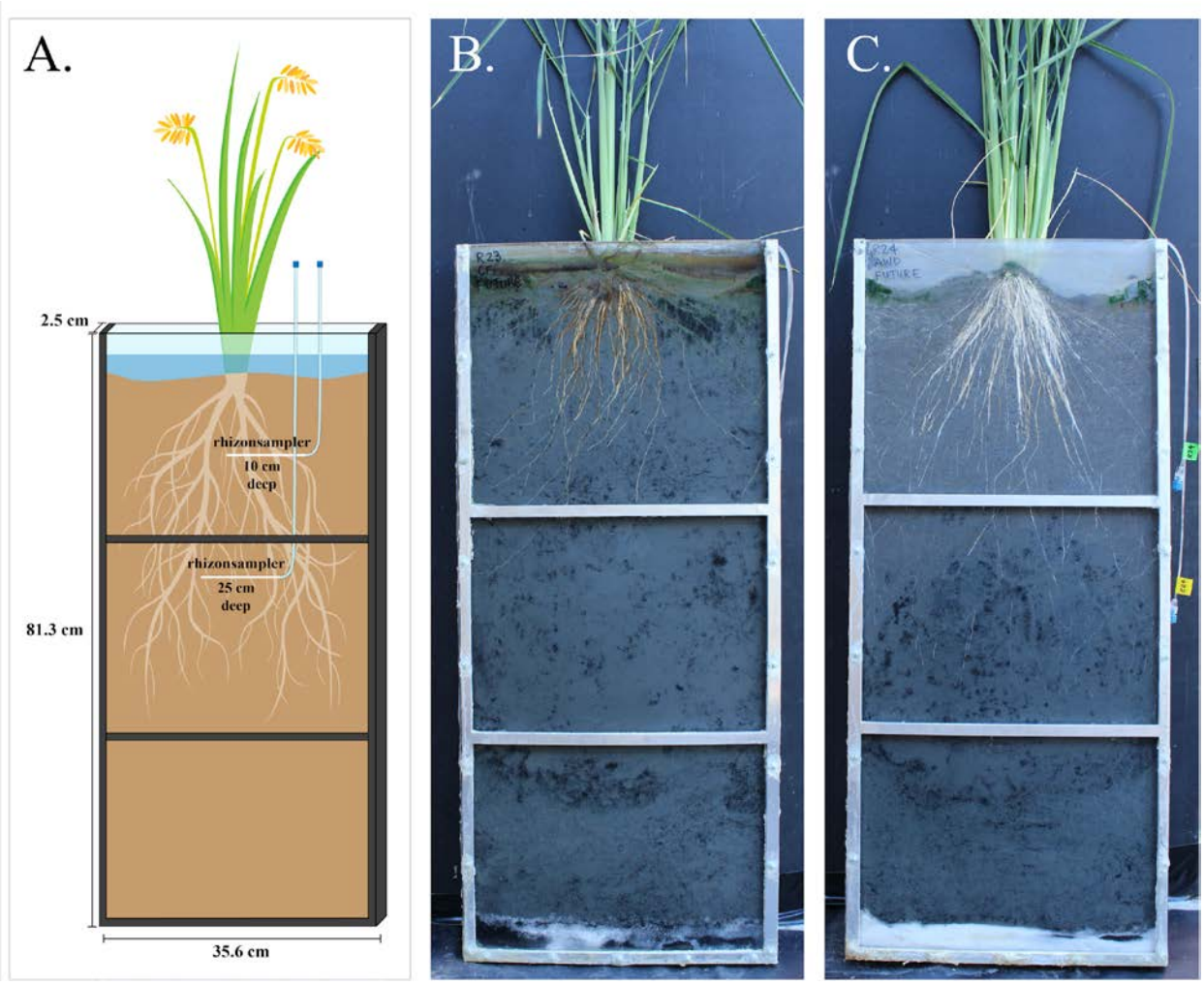


Figure 1: Diagram of rhizobox design (A), example rhizoboxes from the 38 ºC with 850 ppmv CO_2 condition with continuously flooded irrigation (B) and alternate wetting and drying irrigation (C) 88 days after flooding for experiment replicate 1

Rhizoboxes were arranged in a partial randomized block design due to the distinct climate chambers. The chambers were switched halfway through the experiments to decrease biases due

to plant location. For both experiments, the soil was flooded for 24 h before direct seeding (the standard practice in California) of seven rice seeds soaked overnight. After one week, three of the seven seedlings were kept and the rest thinned. After the second week, a single seedling was selected. The rhizoboxes were placed in racks at a 20º angle to encourage root growth along the lower faces of the boxes. The transparent faces were covered in black plastic to minimize soil light exposure. Prior to seeding, urea fertilizer was applied at a rate of 185 kg nitrogen per hectare.

Irrigation management

Throughout the growing season, soils under the AWD condition were flooded for 16 d, dried (via evapotranspiration and downward water drainage) until the soil moisture was 35% volumetric water content (VWC) or less in both climate chambers, and re-flooded for another 16 d (Table 1). The flood duration and soil moisture threshold were selected based on previous research to attempt to sustain or increase rice yields while decreasing grain As.[39,53,55,73,77,78] Soil moisture (soil water potential and VWC) and soil temperature were measured every five minutes for the duration of the experiments using sensors embedded during rhizobox packing (TEROS 12 VWC, Temperature, and EC Sensor and TEROS 21 Soil Water Potential and Temperature Sensor, METER Group, Inc., USA). VWC was measured 10 cm below the soil surface, soil water potential 25 cm below the soil surface, and soil temperature at both depths. The soil moisture time series for both experiments are provided in Figure S4. A longer drainage period was required (11–12 d vs. 4–7 d) to reach the target soil moisture of 35% VWC (10 cm below the soil surface) at the beginning of the season when the rice plants were small and taking up less water. In this manuscript, the phrase “AWD drainage event” indicates a multi-day drying period when no irrigation water was applied, which is sometimes referred to as an AWD “dry-down” in the literature.

Table 1: Alternate wetting and drying drainage period durations and minimum volumetric water content (VWC) prior to reflooding for 16 d for experiment replicate 1

Drain	Duration	Min VWC (33 ºC, 420 ppmv CO_2)	Min VWC (38 ºC, 850 ppmv CO_2)	Growth Stage
1	12 d	35%	26%	Vegetative
2	6 d	29%	16%	Vegetative
3	4 d	11%	14%	Reproductive
4	6 d	20%	14%	Ripening
5	5 d	17%	14%	Ripening

Porewater geochemical analysis

Rhizon samplers (Rhizon Flex 19.21.25, Rhizosphere Research Products, the Netherlands) were embedded horizontally at two depths, 10 cm and 25 cm below the soil surface, during packing (Figure 1A). Porewater was sampled weekly using evacuated, acid-washed glass serum vials (Figure S1A). Samples were prepared for geochemical analyses inside an anoxic glovebag (95% N_2, 5% H_2 atmosphere). Porewater As, Fe, Mn, etc. were quantified using ICP-OES (Thermo ICAP 6300 Duo View Spectrophotometer, Thermo Fisher Scientific Inc., USA) and ICP-MS (Thermo XSERIES 2 Quadrupole ICP-MS and Thermo iCAP Q/Qnova ICP-MS, Thermo Fisher Scientific Inc., USA), after a 1:6 dilution in 2% nitric acid (trace metal grade). Dissolved organic and inorganic carbon were quantified with a total organic carbon analyzer (Shimadzu TOC-L, Shimadzu Corp., Japan). Porewater pH was monitored weekly. The DOC and pH time series are provided in the Supporting Information.

Plant analysis

Above ground plant growth was monitored during the experiments by measuring plant height, tiller number, and panicle number. At harvest, plants were cut 2 cm above the waterline. Tiller diameter was measured at the cut location using calipers. Half of the intact rhizoboxes were rapidly

144 frozen by burial in a large tub of dry ice and quickly transferred to a -20 ºC freezer. For the other
145 half, the front face of the box was carefully removed and soil samples were taken using sterilized
146 spatulas and flash-frozen in liquid nitrogen and then stored at -80 ºC. A pinboard with a 1 cm x 1
147 cm grid of nails and sheet of plastic was carefully pressed into the wet soil to hold the roots in
148 place while the soil was gently washed away to reveal previously obscured roots. The washed root
149 systems were photographed, and then the roots were immediately placed in jars of MilliQ water to
150 prevent desiccation. In the lab, the roots were repeatedly washed with MilliQ water until the rinse
151 water was clean and no visible soil aggregates remained attached to the roots. The wet weight of
152 the roots was recorded, and the root systems were cut in half. The iron plaque on half of each root
153 system was extracted using the dithionite-citrate-bicarbonate (DCB) method.[79] Root and above-
154 ground plant tissues were oven-dried in the dark at 40 ºC until constant weight.
155 After drying, above-ground plant tissues were separated into straw (stems and leaves), panicles,
156 spikelets (unfilled grains), and filled grains. The filled grains were manually dehusked. Plant
157 tissues, including husks, for total As and Cd quantification were finely ground and microwave-
158 digested (MARS 6 Microwave Digestion System, CEM Corp., USA) in 67% nitric acid (trace
159 metal grade) with a 20-25 min ramp phase to 200 ºC (power 1800 Watts; frequency 2450 MHz)
160 and a hold time of 15 min. Diluted extractants were analyzed by ICP-MS; grain extractants were
161 measured using O_2 as the reaction cell gas with a triple quadrupole ICP-MS (8900 Triple
162 Quadrupole ICP-MS, Agilent Technologies, USA) with a detection limit of 0.004 μg L^{-1} for As
163 and 0.0018 μg L^{-1} for Cd. Mass-filtered ^{75}As and ^{78}Se were measured as $^{75}As^{16}O^{+}$ and $^{78}Se^{16}O^{+}$
164 reaction products mass-shifted to m/z 91 and 94 respectively, eliminating spectral interferences
165 from doubly charged rare earth elements. Cd was measured at 112 m/z using He_2 as the reaction

166 cell gas. Every 12 samples, a blank, a spiked sample, and a digested certified reference material
167 (NIST 1515, National Institute of Standards and Technology, USA) were included for quality
168 control. The certified reference recoveries were on average 102% for As and 92% for Cd. Other
169 plant tissues' (straw, husks, and roots) extractants were measured using a double quadrupole ICP-
170 MS (Thermo iCAP Q/Qnova ICP-MS, Thermo Fisher Scientific Inc., USA).

171 To determine grain As speciation, including the presence of thioarsenates, rice grains were
172 extracted following the previously published method that mimics food entering the digestive
173 tract.[31] Briefly, 0.2 g of powdered sample was soaked in 3.2 mL of deionized water and heated for
174 1 h at 80 ºC. Then, the samples were incubated at 37 ºC for 1 h in 10 mL of a phosphate buffer
175 solution (2 mmol·L^{-1} of NaH_2PO_4 + 0.2 mmol·L^{-1} of Na_2-EDTA, adjusted to pH 2 with 5 mol·L^{-1}
176 HCl) and 0.5 mL of pepsin solution (1 g pepsin in 8 mL of 0.1 mol·L^{-1} HCl; ≥ 250 units·mg $solid^{-1}$
177). After that, 1 mol·L^{-1} of $NaHCO_3$ was added to raise the pH to 6, and 2.5 mL pancreatin solution
178 (1.9 g·L^{-1} of 0.1 mol·L^{-1} $NaHCO_3$; 4 × USP specifications) was added. The samples were
179 incubated at 37 ºC for 2 h. The final mass of the solution was adjusted to 15 g with phosphate
180 buffer solution (pH 6), and the samples were filtered (0.45 μm hydrophilic Teflon filters).

181 Arsenic speciation was analyzed immediately by ion chromatography coupled to inductively
182 coupled plasma-mass spectrometry (IC-ICP-MS) using a Metrohm 940 Professional IC Vario with
183 an AG/AS16 IonPac column (Dionex; 2.5 – 100 mmol·L^{-1} of NaOH with gradient elution, a flow
184 rate of 1.2 mL·min^{-1}, and 50 μL injection volume) and ICP-MS (8900 Triple Quadrupole ICP-MS,
185 Agilent Technologies, USA), using O_2 as the reaction cell gas ($^{75}As^{16}O^+$ was measured at m/z 91).
186 The rice flour certified reference material ERM-BC211 was used to evaluate the extraction
187 efficiency and accuracy (extraction recoveries for DMA, iAs, and sum of all As species were
188 101%, 92%, and 100%, respectively). The aqueous reference material TMDA-54 (10-fold diluted;

Environment Canada) was used to assess analytical accuracy (recovery for sum of arsenite, arsenate, and DMA was 100%). Although this method works well for polished rice, low recovery of the sum of As species vs. total As (average 68%) in this study motivated additional tests (see SI), which indicated that enzymatic extraction is not strong enough for full extraction of brown rice samples. DMA recovery is most affected, and the DMA concentrations are, thus, semi-quantitative to qualitative. The sum of species should also be considered semi-quantitative due to the bias toward underestimating DMA. Nevertheless, the enzymatic extraction protocol mimics the process of humans cooking, consuming, and digesting rice, and is the best proxy for human dietary exposure and captures the inorganic arsenic species.

Digital photographs of the rhizoboxes were taken monthly with a DSLR camera (Canon EOS Rebel T7 DSLR camera, Canon USA Inc., USA). The rhizoboxes were briefly removed from the greenhouse chambers and placed in the same location in front of the camera tripod. Photographs were taken outside in the shade using a remote shutter switch to minimize camera motion. A digital color card (Digital Kolor Pro 16:9 Color Calibration and Video Chip Chart, DKG Color Tools, USA) was used to standardize the white balance and color correction, enabling color comparisons of photographs taken at different times during the growing season. Photographs were analyzed using the ImageJ software distribution FIJI with the Root Image Analysis (RIA) plugin.[80] To prepare images for statistical analysis, the background (soil) was removed, the images were converted to binary, any remaining artifacts were removed manually, and the binary images were skeletonized and then analyzed for root traits (total root system length, root system maximum depth, width to depth ratio, etc.) using RIA.

Statistical analysis

All statistics were performed using Python programming.[81] Statistical significance was determined using Welch's t-tests (unpaired) with a *p*-value threshold of 0.05. Two-way analysis of variance (ANOVA) was performed to test for interaction effects between climate and irrigation conditions (see Tables S5 and S6). Correlations between time series were evaluated using Pearson's correlation coefficients.

RESULTS

Rice root response

Rice roots grew more rapidly under the 38 ºC and 850 ppmv CO_2 climate regime (Figure 2A and C, Figure S5). For experiment replicate 1 at two months with CF irrigation, the mean total root system length was 2 times ($p = 0.019$) longer for the 38 ºC and 850 ppmv CO_2 climate compared to the 33 ºC and 420 ppmv CO_2 climate; with AWD irrigation, the mean root system length was 1.3 times longer for the 38 ºC and 850 ppmv CO_2 climate, but this effect was not significant ($p = 0.065$). After three months, the effect of climate was not statistically significant for either irrigation condition, but the mean root lengths were greater by a factor of 1.2 ($p = 0.304$) for CF and a factor of 1.3 ($p = 0.111$) for AWD. Maximum root depth increased with AWD irrigation under both climate conditions (Figure 2B and D); however, this effect was only significant under the 38 ºC and 850 ppmv CO_2 climate condition. After two months under a 33 ºC climate with 420 ppmv CO_2, AWD increased root system maximum depth by 1.3x ($p = 0.059$) and for the 38 ºC climate with 850 ppmv CO_2 by 1.6x ($p = 0.008$) (Figure 2B). After three months, the roots were 1.1x deeper ($p = 0.398$) with AWD for the 33 ºC climate and 1.2x deeper ($p = 0.038$) for the 38 ºC climate (Figure 2D). These robust root systems likely contributed to improved yields due to AWD

under both climate conditions (Table S9).

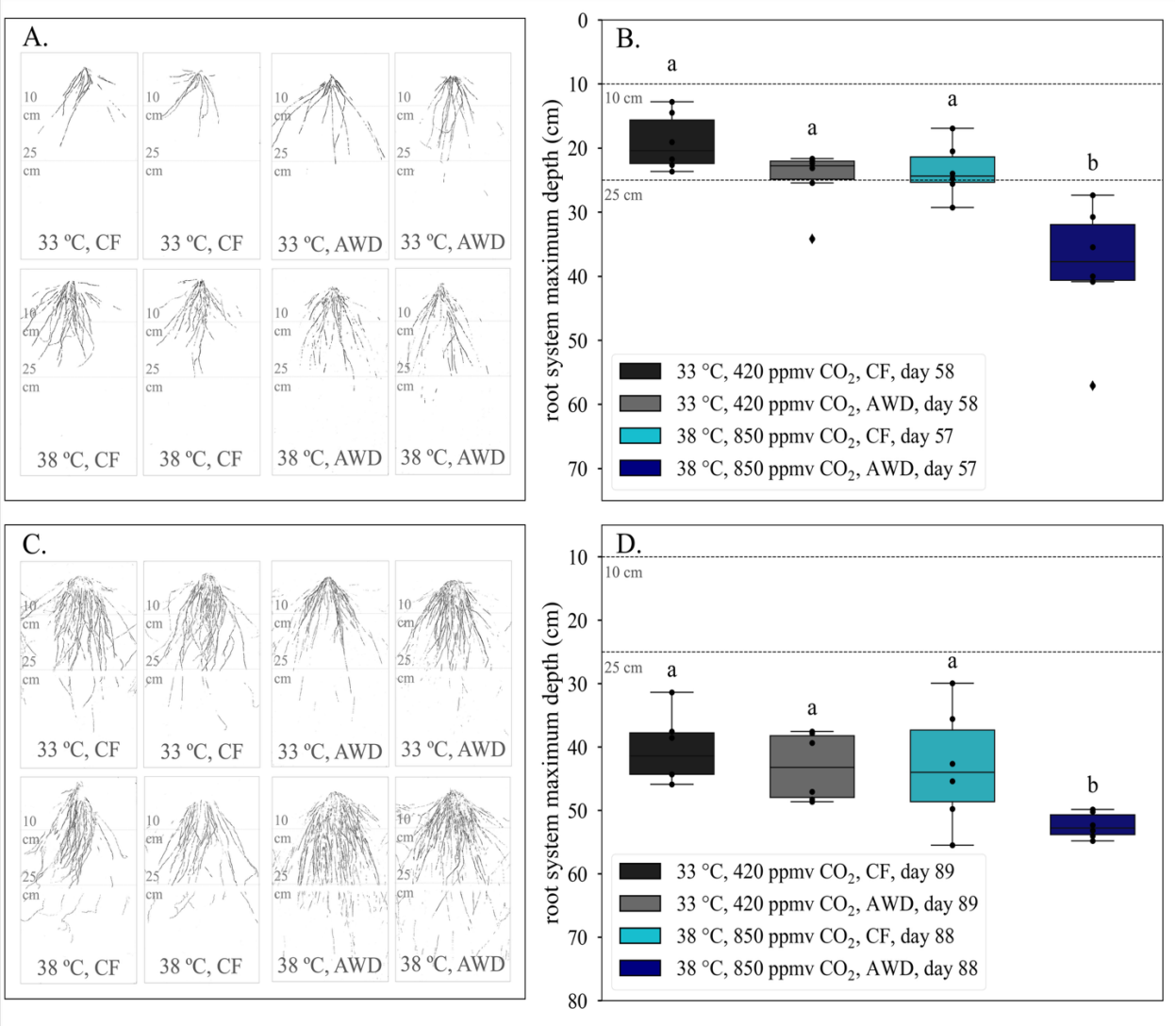


Figure 2: Root skeletons from experiment replicate 1 after 58 d for the 33 ºC with 420 ppmv CO_2 climate and 57 d for the 38 ºC with 850 ppmv CO_2 climate (A); root skeletons after 89 d for 33 ºC with 420 ppmv CO_2 climate and 88 d for the 38 ºC with 850 ppmv CO_2 climate (C); root system maximum depths after ≈2 months (B) and ≈3 months (D); CF = continuously flooded irrigation; AWD = alternate wetting and drying irrigation; different lower-case letters indicate statistical significance ($\alpha = 0.05$), n=6

Porewater arsenic and iron dynamics

Porewater As concentrations decreased immediately following the first AWD drainage event at the 10 cm sampling depth in both experiments (Figures 3A and S6A). However, at the 25 cm sampling depth, dissolved As decreased after the second drainage event (observed on day 56 for both experiments) under the 38 ºC with 850 ppmv CO_2 climate condition and after the third

drainage event (observed on day 77 for experiment replicate 1 (after the completion of experiment replicate 2)) under the 33 ºC climate with 420 ppmv CO_2 (Figures 3B and S6B). The cumulative As concentrations for the entire growing season (calculated using the trapezoidal rule) were approximately: 14.7 mg L^{-1} (integral error estimate ≤ 1.4 mg L^{-1}) for the 33 ºC and 420 ppmv CO_2 climate with CF irrigation and 1.8 mg L^{-1} (error estimate ≤ 0.6 mg L^{-1}) for the 33 ºC and 420 ppmv CO_2 climate with AWD; the 38 ºC and 850 ppmv CO_2 cumulative As concentrations were 15.9 mg L^{-1} (error estimate ≤ 1.2 mg L^{-1}) with CF and 1.0 mg L^{-1} (error estimate ≤ 0.1 mg L^{-1}) with AWD. This corresponds to an 8.2x decrease in cumulative As exposure for the 33 ºC climate with AWD irrigation and a 15.9x decrease for the 38 ºC climate with AWD. Peak porewater As concentrations were higher and occurred earlier under the 38 ºC climate with 850 ppmv CO_2 (Figure 3, Table S4).

Porewater As, Fe, and dissolved organic carbon concentrations were generally higher at the deeper sampling point for all treatments in both experiments (Figures S7 and S8). There was a positive correlation between the As and Fe trends over time (Figures 4, S7, and S8). The Pearson correlation coefficient of the two time-series (based on cubic spline interpolation) was 0.90 (p = 1.12 x 10^{-7}) for the 33 ºC and 420 ppmv CO_2 climate with AWD irrigation and 0.85 (p = 6.91 x 10^{-6}) for the 38 ºC and 850 ppmv CO_2 climate with AWD. With CF irrigation, there was a lag between peak Fe and As concentrations

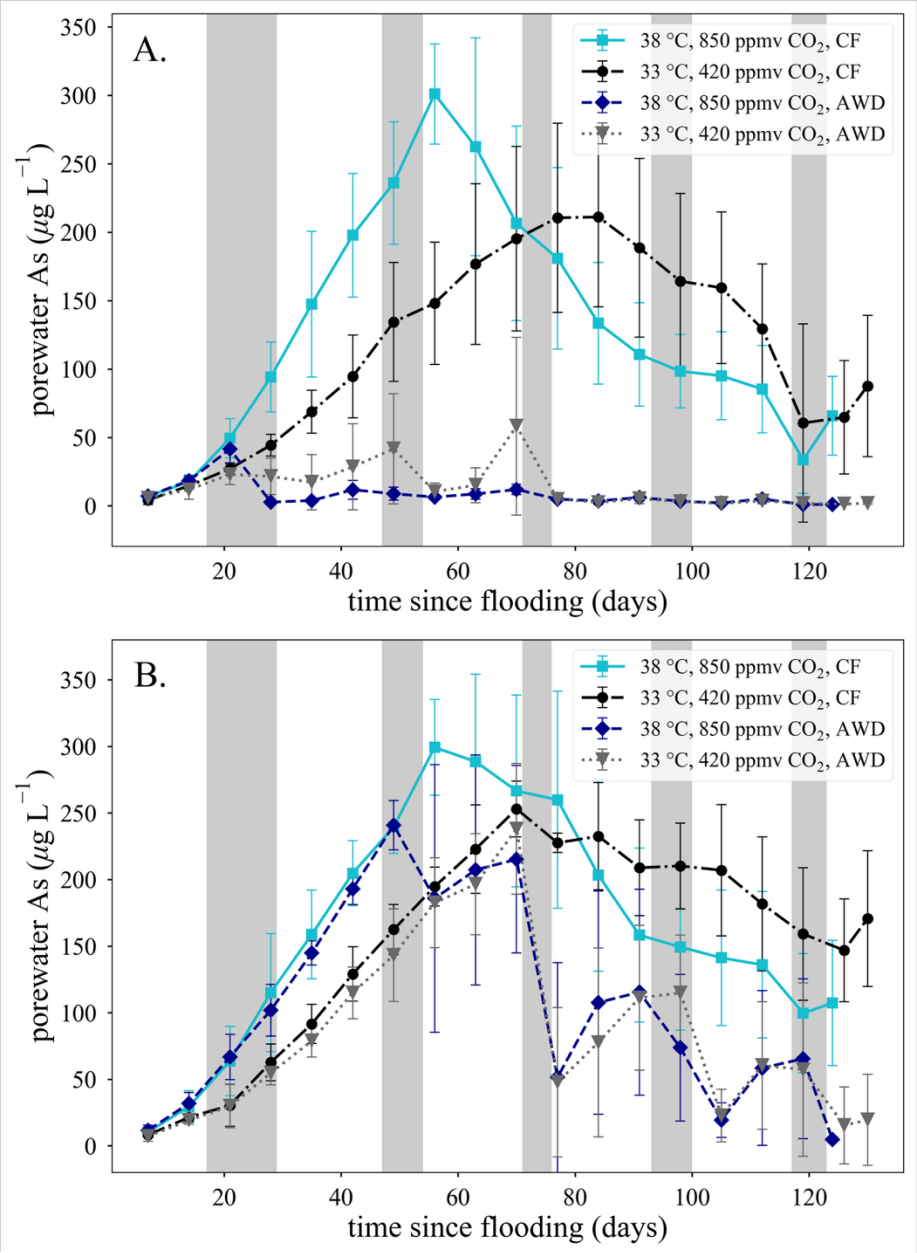


Figure 3: Porewater As concentrations over time 10 cm below the soil surface (A) and 25 cm below the soil surface (B) for experiment replicate 1; CF = continuously flooded irrigation; AWD = alternate wetting and drying irrigation; grey vertical bands indicate drainage events; error bars indicate standard deviation, n=6

resulting in lower correlation coefficients of: 0.69 (p = 0.0011) for the 33 ºC climate and 0.79 (p = 0.00011) for the 38 ºC climate. Accounting for the lag (21 and 14 d offsets for the 33 ºC with 420 ppmv CO_2 and 38 ºC with 850 ppmv CO_2 climates, respectively), the coefficients increased to 0.80 (p = 0.00011) for the 33 ºC climate and 0.88 (p = 3.86 x 10^{-6}) for the 38 ºC climate.

Porewater cadmium dynamics

For replicate experiment 2, porewater Cd concentrations generally decreased for all experimental conditions at both the 10 and 25 cm sampling depths (Figure S9A and C). For replicate experiment 1, porewater Cd concentrations often increased following AWD drainage but never exceeded 1 µg L^{-1} (Figure S9B and

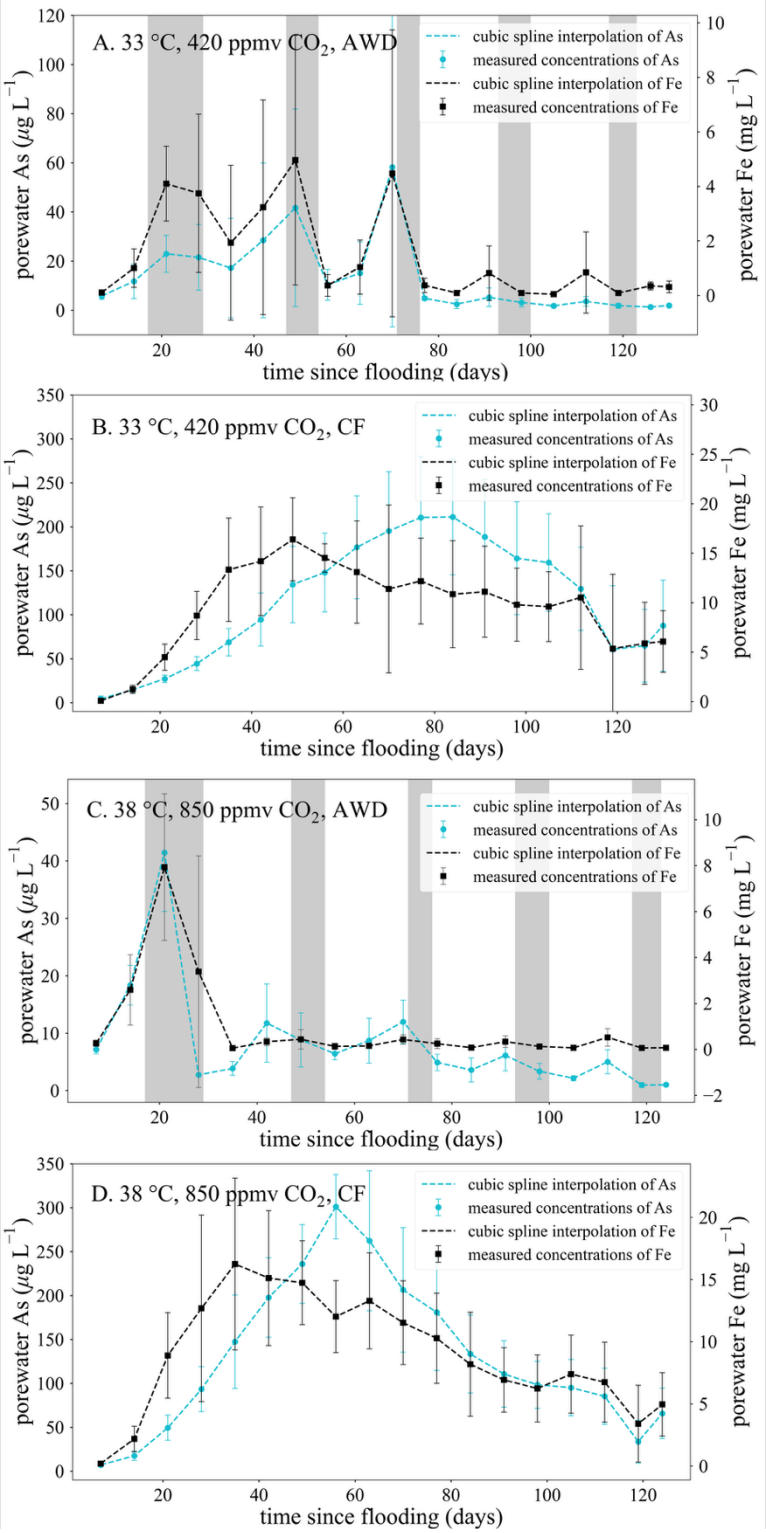
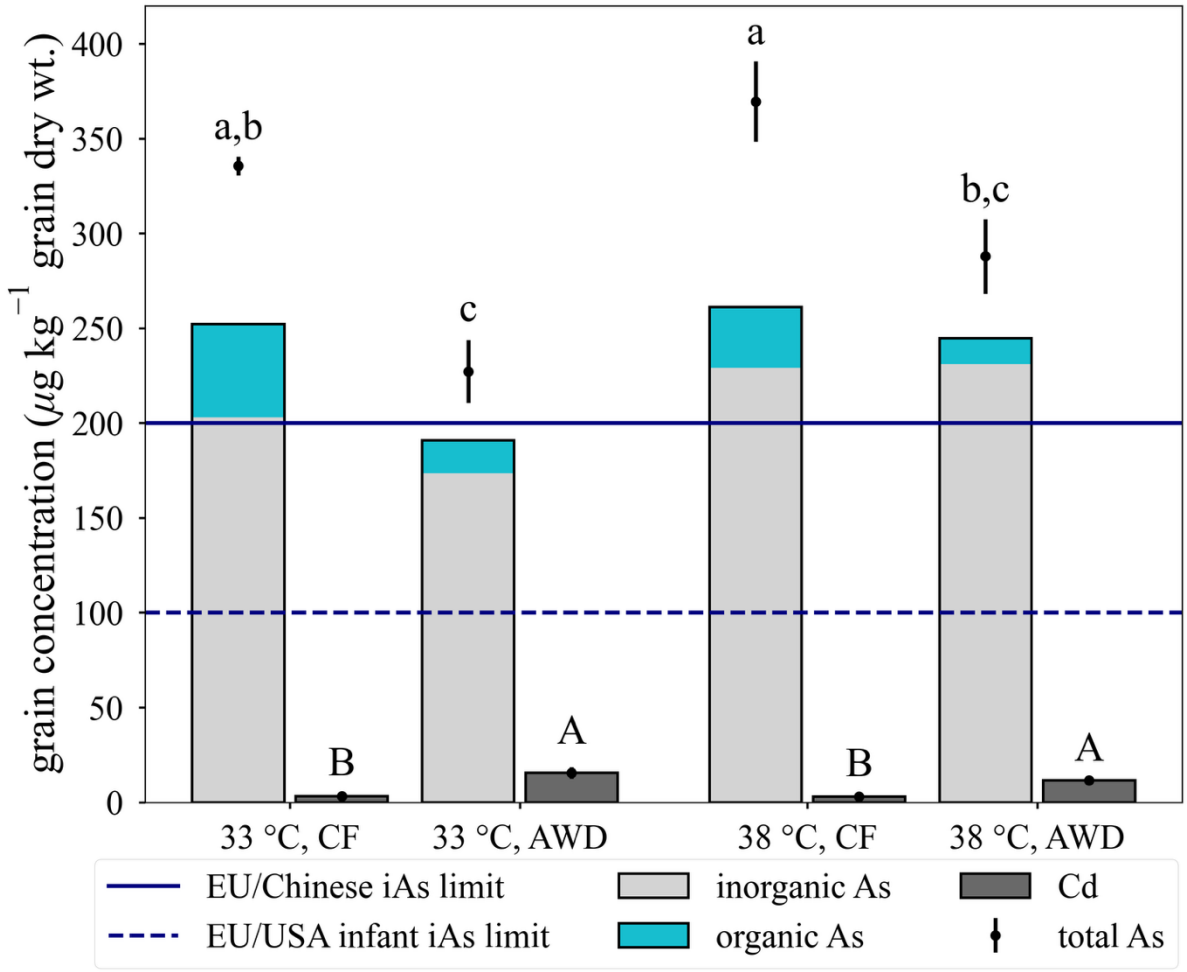


Figure 4: Porewater As and Fe concentrations over time 10 cm below the soil surface for replicate experiment 1; CF = continuously flooded irrigation; AWD = alternate wetting and drying irrigation; grey vertical bands indicate drainage events; error bars indicate standard deviation, n=6

299 D). The seasonal cumulative Cd concentrations were approximately: 6.6 µg L^{-1} (integral error
300 estimate ≤ 1.7 µg L^{-1}) for the 33 ºC and 420 ppmv CO_2 climate with CF irrigation and 25.1 µg L^{-1} (error estimate ≤ 8.8 µg L^{-1}) for the 33 ºC and 420 ppmv CO_2 climate with AWD; the 38 ºC
302 cumulative Cd concentrations were 7.5 µg L^{-1} (error estimate ≤ 3.1 µg L^{-1}) for CF and 17.6 µg L^{-1} (error estimate ≤ 3.3 µg L^{-1}) for AWD.

304 **Grain arsenic and cadmium**

305 Total As concentrations were higher than the sum of As species from enzymatic extraction
306 (Figure 5). The enzymatic extraction does not appear fully efficient for brown rice. This is likely
307 because the amount and activity level of the pepsin used is insufficient to quantitatively recover
308 DMA from whole grain rice (see Table S8); additionally, the technique may miss species such as
309 As(III)-glutathione complexes. The recovery of the sum of species systematically decreased with
310 increasing grain As, and As concentrations were higher for plants grown with CF irrigation. Hence,
311 these results likely underestimate the effect of AWD on grain DMA and should be treated as semi-
312 quantitative to qualitative. Grain inorganic As concentrations did not significantly change due to
313 AWD for either climate (p = 0.12 for the 33 ºC and 420 ppmv CO_2 climate and p = 0.95 for the 38
314 ºC and 850 ppmv CO_2 climate). Hence, significant decreases in total As can be attributed to lower
315 grain DMA and unknown (likely organic) species.



316

Figure 5: Grain inorganic, organic, and total As concentrations, grain Cd concentrations, and inorganic (iAs) regulatory limits for polished rice and infant foods; different lower-case letters indicate statistical significance (α = 0.05) of total As; different upper-case letters indicate statistical significance (α = 0.05) of Cd; error bars indicate standard error, n=6

317 Rice grain total As concentrations were 1.5x (32%) lower (p = 8.1 x 10^{-4}) with AWD under the

318 33 ºC and 420 ppmv CO_2 climate condition, decreasing from an average of 336 to 227 µg kg^{-1}

319 grain dry weight, and 1.3x (22%) lower (p = 0.027) with AWD under the 38 ºC and 850 ppmv

320 CO_2 climate condition, decreasing from 370 to 288 µg kg^{-1} grain (Figure 5). Regardless of

321 irrigation, total grain As concentrations were higher under the 38 ºC climate; however, the effects

322 were not significant. With CF irrigation, grain total As was 1.1x higher (p = 0.22) under the 38 ºC

condition, and with AWD grain total As was 1.3x higher (p = 0.058) under the 38 ºC climate. The 2-way ANOVA results showed no significant interaction effects between climate and irrigation for total As (Table S5).

Rice grain Cd concentrations were higher with AWD irrigation, increasing from 3.1 to 15.4 µg kg^{-1} grain dry weight (p = 0.0026) under the 33 ºC and 420 ppmv CO_2 climate condition and from 3.1 to 11.6 µg kg^{-1} grain ($p = 9.7 \times 10^{-5}$) with AWD under the 38 ºC and 850 ppmv CO_2 condition (Figure 5). However, grain As concentrations were 14.7x–119.7x higher than grain Cd concentrations depending on the experimental conditions. The effect of climate on Cd concentrations was not significant for either irrigation condition.

DISCUSSION

Our results are consistent with earlier findings that report larger and deeper root systems with AWD irrigation.[39,63,64,74,82–84] Song et al. (2020) determined that AWD increases root oxidation activity,[83] and Wang et al. (2022) found that elevated atmospheric CO_2 (800 ppmv) enhanced the effect of AWD on root growth; however, the seedlings were grown for only one month and exposed to elevated CO_2 for only 15 d.[84] Our study reveals that the fertilization effect of CO_2 (and possibly heat stress) may cause more pronounced differences in root growth earlier in the season with plants grown under the 33 ºC and ambient CO_2 climate partially catching up to those grown under the 38 ºC and 850 ppmv CO_2 climate condition after approximately three months.

Although numerous studies confirm that AWD decreases total grain As,[39,40,42,44,46–59,61,62,65,66,78,85–88] few examine the temporal dynamics of As availability in the rhizosphere and, as

344 far as we know, none have tested the efficacy of AWD for decreasing grain As under potential
345 future climate conditions. Our results indicate that near the soil surface, porewater As
346 concentrations decrease rapidly following drainage and only slightly rebound after reflooding for
347 16 d (Figures 3A and S6A) with almost no rebound under the 38 ºC and 850 ppmv CO_2 condition
348 due to more severe drying (Table 1) and differences in the kinetics of arsenate reduction relative
349 to faster arsenite oxidation.[16] Sharp decreases in dissolved As following AWD drainage are likely
350 primarily due to the rapid oxidative precipitation of Fe(III) minerals that adsorb As, which is
351 supported by the simultaneous decreases in porewater Fe concentrations following drainage
352 (Figure 4A and C). Pearson correlation coefficients of 0.90 ($p = 1.12 \times 10^{-7}$) for the 33 ºC climate
353 with AWD irrigation and 0.85 ($p = 6.91 \times 10^{-6}$) for the 38 ºC climate with AWD indicate a strong
354 positive correlation between the porewater As and Fe time series. AWD also decreased Fe plaque
355 formation, presumably due to decreases in aqueous Fe^{2+} (and Mn^{2+}) for oxidative precipitation,
356 decreasing the binding sites for As on root surfaces.
357 Since the rhizoboxes were filled with homogenized topsoil, rather than a combination of
358 subsurface and topsoil, porewater As and Fe concentrations are likely higher than would be
359 expected under field conditions, where less organic matter is present deeper in the soil. The near-
360 surface (10 cm depth) estimated cumulative seasonal As exposure decreased by 8.2x for the 33 ºC
361 climate with AWD and by 15.9x for the 38 ºC climate with AWD. There were corresponding
362 decreases in total grain As concentrations of 1.5x (32%) and 1.3x (23%) under the 33 ºC and 38
363 ºC climates, respectively (Figure 5), which is less pronounced compared to previous studies that
364 report decreases in total As ranging from 41–68% for this rice varietal (M206) grown in
365 Californian soils.[39,54] The difference may be due to the artificial constraints of the rhizoboxes

366 forcing roots to grow deeper than they would under field conditions. The deeper roots likely
367 accessed permanently saturated zones where the As is more labile, especially under the 38 ºC
368 climate with 850 ppmv CO_2. This interpretation was corroborated by visible roots deep in the
369 rhizoboxes where the soil appeared gleyed for both AWD treatments. In these saturated zones,
370 root Fe plaque was clearly visible. This may also explain why the decrease in total grain As due to
371 AWD was smaller under the 38 ºC climate condition (1.3x vs. 1.5x under the 33 ºC condition)
372 even though the plants were exposed to less porewater As at the 10 cm sampling depth. However,
373 quantitative correlations between maximum root depth at three months and total grain As were not
374 significant (Table S10). In a previous pot study with CF irrigation, +5 ºC warming increased total
375 grain As 1.5x.[35] Our experiments with the same warming scenario led to a 1.1x increase in total
376 grain As with CF and a 1.3x increase with AWD.
377 In the present study, decreases in grain inorganic As due to AWD were not statistically
378 significant (p = 0.12 for the 33 ºC climate and p = 0.95 for the 38 ºC climate). Hence, decreases in
379 total As are due to lower (estimated) DMA and unknown species concentrations. Inorganic As
380 concentrations in the brown rice exceeded the limit for infant foods (100 µg kg^{-1} grain dry weight)
381 for all experimental conditions, and the only condition that met the limit for polished rice for adults
382 was the 33 ºC climate with AWD. Although polished grain data are not included in this study,
383 based on these findings we speculate that AWD may be necessary to achieve current regulatory
384 standards even under today's climatic conditions for rice grown in 'uncontaminated' soils, and
385 climate change experiments in more contaminated soils including polished grain data are merited.
386 Our findings suggest that AWD may have more pronounced benefits for rice plants, mitigating
387 straighthead disease and enhancing yields, compared to minimizing grain inorganic As.

In contrast to As, AWD often increases porewater and grain Cd concentrations because aerobic conditions encourage Cd mobilization in the rhizosphere.[8,33,40,49] The porewater Cd concentrations were low in both experiments and the trends were inconsistent (Figure S9), possibly due to small changes in soil and rhizosphere process heterogeneity. For replicate experiment 2, porewater Cd concentrations decreased over two months for all experimental conditions (Figure S9A and C). However, during replicate experiment 1, porewater Cd concentrations generally increased following drainage events, as expected (Figure S9B and D). As a result, grain Cd concentrations were higher due to AWD but were modest in comparison to the decreases in grain As (Figure 5); under the 33 ºC and ambient CO_2 climate condition, grain Cd increased from 3.1 to 15.4 µg kg^{-1} grain dry weight (p = 0.0026), and under the 38 ºC and 850 ppmv CO_2 climate condition, grain Cd increased from 3.1 to 11.6 µg kg^{-1} grain ($p = 9.7 \times 10^{-5}$) with AWD. These values are similar to those reported in other studies with low soil Cd concentrations and are well below the FAO/WHO dietary limit of 400 µg Cd kg^{-1} grain.[54,89] Although it is possible that higher soil Cd concentrations could amplify the effects of AWD and future climate conditions on grain Cd, the effect of AWD is already quite large. Using a quantitative model based on 17 field studies, Carrijo et al. (2022) demonstrated that Cd is more sensitive to AWD than As.[39] When soil Cd concentrations are high, they suggest avoiding drying during the early reproductive stage to decrease Cd concentrations in rice grain, and soil amendments in combination with AWD can decrease both As and Cd simultaneously.[44,85]

Our results indicate that AWD has the potential to mitigate grain As accumulation even under severe future climate conditions. However, AWD does not work as well under simulated future climate conditions—decreasing grain As by 1.3x (22%) compared to the 1.5x (32%) decrease

observed under the 33 ºC and 420 ppmv CO_2 climate—despite more rapid soil drying and limited near-surface porewater As for the 38 ºC condition. Importantly, compared to CF irrigation under the 33 ºC climate, AWD under the 38 ºC climate decreased grain As by 1.2x. This suggests that adopting AWD in the future could compensate for temperature-induced increases in grain As. The temporal dynamics of As mobilization in the rhizosphere are strongly depth-dependent, and enhanced root growth due to atmospheric CO_2 fertilization may further impact the muted effect of AWD on grain As under future climate conditions. A longer drainage period and/or more frequent drainage may be needed in the future to further improve the effectiveness of AWD for limiting grain As concentrations. Although AWD can increase grain Cd concentrations, this study provides additional evidence that the trade-off between decreasing As and increasing Cd is worth it when rice is grown in soils with low Cd concentrations.[52,54,66,85] Although larger scale climate and AWD experiments are needed, our findings provide optimism for protecting future rice production and human health using irrigation management.

SUPPORTING INFORMATION

Additional supporting tables, figures, and description of rhizoboxes (.pdf)

All root images (both raw (.cr2 and .JPG) and skeletonized (.tif))

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