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Short Communication

# Irrigation management for arsenic mitigation in rice grain: Timing and severity of a single soil drying

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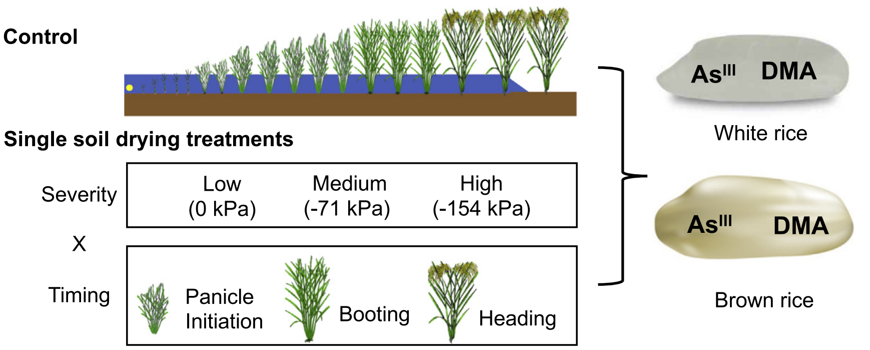
## HIGHLIGHTS

- Continuously flooded irrigation favors the accumulation of arsenic in rice grain.
- Treatments with one soil drying period differing in timing and severity were tested.
- Across all timings, severe soil drying (≤−71 kPa) decreased total As concentration.
- However, inorganic As (the most toxic to humans) not always decreased.
- Irrigation management affects both total As and As speciation within rice grain.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The accumulation of arsenic (As) in rice grain is a public health concern since As is toxic to humans; in particular, inorganic As can cause many chronic diseases including cancer. Rice crops are prone to accumulating As, in part, due to the anaerobic soil conditions triggered by the traditional continuously flooded irrigation practice. The objective of this study was to determine how the severity and the timing (i.e. crop stage) of a single soil drying period impact total As concentration and As speciation within the rice (both white and brown) grain, compared to a continuously flooded (CF) control. Drying the soil until the perched water table reached 15 cm below the soil surface (same severity as in the "Safe Alternate Wetting and Drying"), which in this study corresponded to a soil (0–15 cm) water potential of ~0, did not decrease grain As concentrations, regardless of timing. Drying the soil to Medium Severity [MS: soil (0–15 cm) water potential of −71 kPa] or High Severity [HS: soil (0–15 cm) water potential of −154 kPa] decreased total As by 41–61%. However, inorganic As did not always decrease because the severity and the timing of soil drying affected As speciation within the grain. Overall, the soil had to be dried to HS and/or late in the growing season (i.e., at booting or heading instead of at panicle initiation) to decrease inorganic As concentration in the rice grain. This study indicates that the imposition of a single soil drying period within the growing season can mitigate As accumulation in rice grain, but it depends on the severity and timing of the drying period. Further, irrigation management affects As speciation within the rice grain and this must be considered if regulations on inorganic As are based on a percentage of total As measured.

## 1. Introduction

Rice is the primary staple food for more people on Earth than any other crop and provides one quarter of the global calorie intake

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(GRiSP, 2013). However, rice can be a significant route of human exposure to arsenic (As), especially in populations with high rice consumption (Meharg, 2004; Bhowmick et al., 2018). Different forms of As are found in rice grains, the most common being the inorganic species arsenite ($As^{III}$) and arsenate ($As^{V}$) and the organic species monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). While the toxicities of the organic As species are considered to be low (Hirano et al., 2004), exposure to inorganic As is associated with many types of cancer, in addition to other non-carcinogenic diseases such as diabetes and hypertension (Bjørklund et al., 2017). Inorganic As accumulates in the bran, causing paddy and brown rice to have higher inorganic As concentration than white rice (Sun et al., 2008). Maximum levels for inorganic As of 0.35 and 0.2 mg $kg^{-1}$ in paddy and white rice, respectively, have been adopted by CODEX, a joint commission of the World Health Organization and the Food and Agriculture Organization (CODEX, 2018).

Arsenic in rice grain originates from the soil, where it can be naturally present (e.g., parent rock) or carried over to via irrigation (i.e., water with high As levels) or other sources (e.g., arsenical pesticides, urban residue) (Kumarathilaka et al., 2018). Arsenic tends to accumulate in rice for two reasons. First, the uptake of $As^{III}$ and, in part, MMA are mediated by the silicon uptake pathway; rice being a silicon accumulator is therefore naturally effective in taking up As from the soil (Suriyagoda et al., 2018). Second, rice is commonly grown under flooded conditions for most of the growing season. Anaerobic soil conditions increase As bioavailability in the soil because it triggers the reduction of $As^{V}$ to $As^{III}$, which is more mobile in the soil, and of $Fe^{III}$ to $Fe^{II}$, dissolving iron hydro(oxides) that bind to As and, subsequently, releasing As to the soil solution (Meharg and Zhao, 2012). In addition, the high irrigation input to rice contributes to As building up in the soil if the irrigation water has high As levels (Kumarathilaka et al., 2018).

Since soil flooding is a major contributor to As accumulation in rice grains, irrigation practices that include one or more periods of soil drying within the growing season [e.g., alternate wetting and drying (AWD), intermittent flooding, mid-season drain] have been proposed as mitigation strategies (Bakhat et al., 2017). However, the impact of these irrigation practices on grain As concentration is highly variable, with decreases of 0 to 90% in total grain As being reported (Arao et al., 2009; Linquist et al., 2015; Honma et al., 2016; Yang et al., 2016; Norton et al., 2017a; Carrijo et al., 2018). This variability may be attributed, at least in part, to differences in the severity and timing (i.e. crop stage) of soil drying.

The severity of soil drying affects soil redox potential (Eh), which is intrinsically related to soil As bioavailability, and subsequently As uptake. Grain As concentration decreases sharply with an increase in soil Eh from −200 to −100 mV and tends to plateau at very small concentrations (<0.02 mg $kg^{-1}$) as soil Eh increases above 0 (Honma et al., 2016). Carrijo et al. (2018) found that total grain As concentration decreased with increasing soil drying severity, although drying the top soil (0–15 cm) to a water potential lower than −33 kPa did not translate into a further decrease in grain As concentration. However, their study was limited to total As concentrations and, given that As speciation may be affected by soil drying (Yamaguchi et al., 2014), their conclusions may not apply to all As species present in the grain.

The effect of timing of soil drying on grain As concentration has been scarcely investigated, with the exception of a few pot studies (e.g., Arao et al., 2009; Li et al., 2009). Soil drying would be most effective in minimizing grain As concentration if imposed when As uptake is highest under flooded conditions. However, predicting temporal As uptake is a difficult task considering the many variables regulating As availability and uptake. Reports that the expression of Lsi1, a root transporter involved in the uptake of $As^{III}$, is enhanced around the heading stage (Yamaji and Ma, 2007; Ma et al., 2008), suggest that this could be a key stage for As uptake. In contrast, Li et al. (2015) reported that of the total As present in aboveground tissues at harvest, 64% had been taken up at the jointing (~panicle initiation) and booting stages, and they attributed that to enhanced nutrient uptake and root size during this period. In addition, As uptake is strongly influenced by the formation of iron plaques on the surface of rice roots, and their formation and capacity of sequestering As is dependent on crop stage (Garnier et al., 2010; Awasthi et al., 2017; Yu et al., 2017). For example, Mei et al. (2012) found that plants at the bolting (~booting) stage showed higher root radial oxygen loss and higher root porosity than plants at the tillering stage, and this translated into higher As sequestration in the root plaque and lower As uptake.

In this study, we sought to quantify how the severity and the timing of soil drying impact total As and As speciation within the rice grain. We hypothesized that grain arsenic concentration (total and individual species) would decrease with increasing soil drying severity independent of crop stage.

## 2. Material and methods

### 2.1. Study site characteristics

A field experiment was conducted at the Rice Experiment Station (39°27′47″N, 121°43′35″W) in Biggs, California, USA, during the summer of 2016. The soil at the site is a Vertisol, comprised of fine, smectitic, thermic, Xeric Epiaquerts and Duraquerts, with a soil texture of 29% sand, 26% silt and 45% clay, a pH of 5.3, 1.06% organic C and 0.08% total N (Pittelkow et al., 2012). Total As concentration in the soil was 3.85 mg $kg^{-1}$ (Carrijo et al., 2018) and in the irrigation water it averaged 1 μg $L^{-1}$ in the growing season. Total As in irrigation water was measured by collecting water samples from the main irrigation canal on four sampling dates (July 8th and 25th, August 9th and September 16th). Samples were immediately filtered [through glass microfiber filter paper (Whatman GF/F)] and acidified with nitric acid (67–70%, trace metal grade) to a pH of 2 prior to storage at 4°C. Total As in water was quantified by inductively coupled plasma (ICP) mass spectrometry (MS), following the same methodology described for rice grains in Section 2.6.2. The climate is Mediterranean, and the total precipitation and average daily temperature over the growing season (May through October) was 10.1 mm and 21.7° C, respectively (CIMIS, 2018).

### 2.2. Treatments

There were ten irrigation treatments: a continuously flooded (CF) control, which was maintained flooded (i.e., standing water maintained at ~12 cm above the soil surface) from sowing to three weeks before harvest (pre-harvest drain), and nine treatments in which a single soil drying period was imposed, and that represented a combination of three timings and three severities of soil drying. The three timings, which determined the onset of the soil drying period, were: at panicle initiation, during booting and at 50% heading (this extended into the early grain filling period for most treatments). We did not include soil drying timings that were earlier than panicle initiation due to the risk of potential for high fertilizer nitrogen losses (LaHue et al., 2016). The severities were: Low Severity (LS - reflooded when the perched water table reached 15 cm below the soil surface), Medium Severity (MS - reflooded when the soil volumetric water content at the 0–15 cm soil depth reached 35%), and High Severity (HS - reflooded four days after the MS treatments). The choice on severity treatments aimed at representing a wide range of severities, from the LS, which is the severity used in a common form of AWD known as “Safe-AWD” (widely adopted in some Asian countries and considered to not limit rice yields) (Bouman et al., 2007; Lampayan et al., 2015), to the HS, which is considered to limit rice yields (Carrijo et al., 2018). All soil drying treatments underwent a single drying period according to their respective timing/severity, and except for this period, followed the same water management as in the CF. Within each timing, all plots started drying together and plots of the same severity were reflooded at the same time when, on average, the targeted severity was reached across plots. No precipitation occurred during any of the drying events. Detailed information about the irrigation treatments and general management practices are reported in Table 1.

### 2.3. Experimental design and general management practices

The experiment was laid out in a completely randomized design with four replications. Plots were comprised of polyvinyl chloride cylinders (30 cm in height and 76 cm in diameter – total area of 0.46 $m^2$) that were buried 20 cm deep in the soil inside a rice field (0.3 ha basin) and 2 m apart. A fertilizer blend of mono-ammonium phosphate, urea, ammonium sulfate and muriate of potash was banded at 5–7 cm below the soil surface, which provided a total of 171, 45 and 25 kg $ha^{-1}$ of N, $P_2O_5$ and $K_2O$, respectively. Following fertilization, seeds of the medium grain variety M-206 were broadcasted at the rate of 168 kg $ha^{-1}$, and floodwater was applied. Pesticides were applied to all cylinders as necessary.

Irrigation was managed in individual cylinders by having two holes drilled in each cylinder just above the soil surface (which could be plugged or unplugged for holding or draining water, respectively) and a drip irrigation line mounted on top of the cylinders to supply water (same irrigation source as for the rest of field) when needed. At the start of each soil drying treatment, the main field was drained along with the desired treatment cylinders. The cylinders destined to be kept flooded were plugged and floodwater was maintained using irrigation from the drip line. Except for when there was a soil drying treatment, the main field was maintained flooded as in the CF to maintain plant growth outside the cylinders, thus preventing border effects. In addition, whenever the field was flooded (i.e. all treatments flooded), cylinders were kept unplugged and, except for during the first three weeks after sowing, floodwater height was maintained above the top of the cylinders (i.e., ~12 cm above the soil surface) to allow floodwater exchange between cylinders and field.

### 2.4. Soil moisture measurements

Soil volumetric water content (VWC) at 0–15 cm depth was monitored using capacitance sensors (10HS, Decagon Devices Inc., Pullman, WA) connected to data loggers (Em50, Meter Group Inc., Pullman, USA). The sensors were installed vertically in the soil with their centers at 7.5 cm soil depth, and had a volume of influence of 1 L, which spanned from 0.5 to 14.5 cm soil depth. Soil water potential (WP) at 0–15 cm depth was monitored using electrical resistance sensors (Watermark 200SS, Irrometer Co Inc., Riverside, CA) connected to data loggers (900 M Monitor, Irrometer Co Inc., Riverside, CA). The sensors were installed vertically in the soil with their centers at 7.5 cm soil depth. One VWC and one WP sensor was installed in all soil drying treatment plots and, for comparison, one VWC and one WP sensor was installed in one of the CF plots. The perched water table (PWT) was measured at the end of the drying period in all the LS treatment plots using perforated tubes. In each LS treatment plot, a 30 cm long, 5 cm diameter polyvinyl chloride tube perforated with 1 cm diameter holes spaced approximately 2 cm apart was inserted 25 cm deep into the soil after drilling a hole of the exact same diameter.

**Table 1**
Summary of management practices.

| Crop development and general management practices | Date | DAS |
|---|---|---|
| Fertilization | May 26 | −1 |
| Sowing and initial soil flooding | May 27 | 0 |
| Panicle initiation[a] | Jul 11 | 45 |
| 50% heading[b] | Aug 15 | 80 |
| Pre-harvest drain | Sep 21 | 117 |
| Harvest | Oct 12 | 138 |
| **Water management in soil drying treatments** | **Date** | **DAS** |
| Start of panicle initiation drying period[c] | Jul 12 | 46 |
| LS reflooded | Jul 15 | 49 |
| MS reflooded | Jul 22 | 56 |
| HS reflooded | Jul 26 | 60 |
| Start of booting drying period | Jul 28 | 62 |
| LS reflooded | Jul 29 | 63 |
| MS reflooded | Aug 5 | 70 |
| HS reflooded | Aug 9 | 74 |
| Start of heading drying period | Aug 15 | 80 |
| LS reflooded | Aug 17 | 82 |
| MS reflooded | Aug 26 | 91 |
| HS reflooded | Aug 30 | 95 |

Abbreviations: DAS = days after sowing; LS = low severity; MS = medium severity; HS = high severity.

[a] Determined according to the University of California Agriculture and Natural Resources degree day model for California rice varieties available at http://rice.ucanr.edu/Degree_Day_Model/.

[b] When 50% of the panicles in the field had at least partially exserted from the boot.

[c] The start day of a soil drying period was considered the day when the perched water table was at the soil surface.

### 2.5. Yield, yield components and grain milling for As quantification

When plants were mature, all plants were manually harvested from within each plot. The total number of tillers was counted and the number of panicles was counted from 50 representative tillers to estimate the percentage of unproductive tillers. Grain and straw were dried at 65 °C until constant weight for the determination of yield and harvest index, and 1000 grains were counted using a seed counter (Model 750-2C, International Marketing and Design Corp., San Antonio, TX) and weighed. Yield and 1000-grain weight were adjusted for 14% moisture. Brown rice was obtained by removing the husk from paddy grain using a laboratory dehusker (Model FC2K-Y, Yamamoto Co. Ltd., Yamagata, Japan). White rice (i.e. polished rice) was obtained by removing the husk and the bran from paddy grain using a laboratory mill (Paz-1/DTA, Zaccaria USA, Anna, TX). Grains were ball milled to pass a 250 μm sieve and stored in the dark at 4 °C prior to As analysis.

### 2.6. As quantification

#### 2.6.1. Chemicals

All water used for analysis was 18.2 MΩ·cm (Barnstead Nanopure). Trace metal grade nitric acid (67–70%), ammonium phosphate dibasic (≥99%) and ammonium hydroxide (28–30%) were from Fisher Chemical (USA). Stock standards of arsenite (1001 mg $L^{-1}$) and arsenate (998 mg $L^{-1}$) were from Spex Certiprep (USA) and of DMA (≥98%) and MMA (≥98.5%) were from ChemService (USA). Rice flour certified reference material (CRM 1568b) was from National Institute of Standards and Technology (NIST).

#### 2.6.2. Total As

Total As in grains was determined following the method of Sun et al. (2008) with some modifications described as follows. Samples of 0.5 g (two analytical replicates per plot) were digested in glass digestion tubes by adding 5 mL of nitric acid and allowing it to dissolve overnight at room temperature. Samples were further digested in a heating block at 105 °C until the cessation of a brown fog, and then at 120 °C until complete dryness. The ash was re-dissolved with 10 mL of 0.28 mol $L^{-1}$ nitric acid and filtered using a syringe filter (0.45 μm), taking care to discard the first 1 mL of the filtrate. The extract was then diluted 5-fold with water.

Total As in grain samples (following the digestion method described above) and irrigation water samples (as collected in the field, without further preparation) was quantified by inductively coupled plasma mass spectrometry (ICP-MS 7900, Agilent Technologies, Santa Clara, CA, USA) with a detection limit of 0.01 μg $L^{-1}$. As was monitored at $m/z$ of 75 and selenium was also monitored ($m/z$ 77, 78 and 82) to check for polyatomic $^{40}Ar^{35}Cl$ interferences on $m/z$ 75.

### 2.6.3. As speciation

As speciation in grains was determined as in FDA (2012). In brief, samples of 1 g (two and three analytical replicates per plot for white and brown rice, respectively) were digested in plastic centrifuge tubes with 4 mL of 0.28 mol $L^{-1}$ nitric acid at 95 °C for 90 min. The digested sample was centrifuged at 5858*g* for 15 min and the supernatant was neutralized with the mobile phase (10 mmol $L^{-1}$ ammonium phosphate dibasic, pH of 8.25 adjusted with ammonium hydroxide) and ammonium hydroxide to a target pH of 6.0 to 8.5. The resulting solution was filtered (0.45 μm nylon) prior to analysis.

Four As species ($As^{III}$, $As^{V}$, DMA and MMA) were quantified by high performance liquid chromatography (HPLC 1200 series, Agilent Technologies) coupled with ICP-MS 7900. Arsenic species (elution order: $As^{III}$, DMA, MMA and $As^{V}$) were separated using an anion-exchange column (Hamilton PRP-×100, 250 mm × 4.1 mm × 10 μm) with isocratic mobile phase at 1.0 mL $min^{-1}$ and then quantified by ICP-MS following the same procedure as for total As, but with a detection limit of 0.1 μg $L^{-1}$. The percentage of inorganic As in grains (grain inorganic As%) was calculated as in Eq. (1):

$$\textit{Grain inorganic As\%} = 100 \times \frac{As^{III} + As^{V}}{As^{III} + As^{V} + DMA + MMA} \tag{1}$$

where $As^{III}$, $As^{V}$, DMA and MMA are the grain concentrations of these species as quantified by the As speciation analysis.

### 2.6.4. Quality control

In both total and speciation analyses, at least one blank, one fortified sample (for the total As analysis, $As^{V}$ was spiked at 0.1 mg As $kg^{-1}$ grain; for the speciation analysis, all four species were spiked at 0.1 mg As $kg^{-1}$ grain), and one reference material (CRM 1568b) were included with every 10 samples analyzed. Analytical replicates were accepted when their coefficient of variation was within 15%. For each plot, mass balance was performed between the sum of the four species determined by HPLC-ICP-MS and the total As determined by ICP-MS. Sample grain moisture was measured and As concentrations are presented on a dry mass basis. Recoveries of fortified samples and reference material and mass balances were calculated as described in FDA (2012) and are presented in Table S1, Appendix A.

### 2.7. Statistical analyses

All statistical analyses were performed in R software (R Core Team, 2016) and all variables were fit to a linear model before being subjected to analysis of variance (ANOVA). A one-way ANOVA was performed on grain As concentration (separately for brown and white rice and total As and individual As species), yield and yield components, with treatment as a fixed effect, followed by Tukey means separation. A two-way ANOVA was performed on soil VWC and WP with timing and severity (as well as their interaction) as fixed effects, followed by Tukey means separation; the CF control was excluded from this analysis since these measurements were taken in only one CF plot. A one-way ANOVA was performed on PWT with timing as a fixed effect, followed by Tukey means separation. Grain As concentration data from brown and white rice were combined and a two-way ANOVA was performed on grain inorganic As% with treatment and grain milling (including their interaction) as fixed effects. Grain milling means were separated by Tukey means separation. The effect of treatment was analyzed using a set of single-degree-of-freedom orthogonal contrasts to provide information on the factorial part of the experiment (i.e. main effects of timing and severity and their interaction), and the Sidak correction was used to control the familywise error rate. The set of contrasts excluded all LS treatments for reasons discussed in the results Section 3.4.

## 3. Results

### 3.1. Soil moisture in the soil drying treatments immediately before reflooding

To ensure that there were no confounding effects between timing and severity of soil drying, we tested the hypothesis that soil moisture measured at the end of the drying period was the same across treatments of the same severity (Table 2). Although there were small differences in the number of drying days required to achieve a desired soil moisture, soil WP was the same within each severity (i.e., independent of timing) and increased in the order: HS < MS < LS, as expected. Similarly, there was no difference in soil VWC within each severity except for the MS ($p = 0.015$), where soil VWC was lower in the treatments dried at panicle initiation than at booting. The PWT, measured only in the LS treatments, was the same across all timings and averaged 17 cm below the soil surface.

Given the inaccuracy of the soil WP sensor in the 0 to −10 kPa range (Irmak and Haman, 2001; Shock and Wang, 2011), we assume that any value in this range is ~0 kPa (Table 2). Averaged across timings, soil VWC was 48%, 36% and 33% and soil WP was −1 (~0), −71 and − 154 kPa at the end of the drying period in the LS, MS and HS treatments, respectively (Table 2). For comparison, soil VWC and WP in the CF control averaged 48% and − 9 kPa (~0) throughout the season, excluding the pre-harvest draining period. The severities achieved in the LS and MS treatments were close to what was targeted (i.e., PWT of −17 cm vs. −15 cm targeted for LS and VWC of 36% vs. 35% targeted for MS). Averaged across timings, the drying period lasted 2, 10 and 14 days in the LS, MS and HS treatments, respectively.

### 3.2. As in white rice

In all the LS treatments, the concentration of total As (Fig. 1A) and various As species (Fig. 1C) was similar to the CF control. For both MS and HS treatments, independent of timing, total As concentration decreased by 42–61% compared to the CF control. Of the four As species (i.e., organic MMA and DMA, and inorganic $As^{III}$ and $As^{V}$) quantified in this study, only $As^{III}$ and DMA were detected in white rice. Drying the soil to HS (independent of timing) or to MS at booting (but not at other timings) decreased $As^{III}$ concentration by 31–46% compared to the CF control. DMA concentration decreased by 61–71% when the soil was dried down to MS or HS at panicle initiation or booting, but not at heading, compared to the CF control.

### 3.3. As in brown rice

Similar to what was observed for white rice, total As (Fig. 1B) and As speciation (Fig. 1D) in brown rice were similar in the LS and CF treatments. Total As concentration in brown rice decreased in all the MS and HS treatments, compared to the CF control, similar to what was observed for white rice. However, the decrease was dependent on the timing of the drying period. Drying the soil to HS at booting decreased total As by 59%, whereas drying the soil to MS at panicle initiation or heading decreased total As by 41–42%. There was no MMA or $As^{V}$ detected in brown rice of any treatment. In the MS and HS treatments, $As^{III}$ concentration was 41–56% lower than in the CF control at booting or heading, but not at panicle initiation. DMA concentration decreased by 71–81% when the soil was dried down to MS or HS at panicle initiation or booting, but not at heading, compared to the CF control. Averaging across all treatments, total As, $As^{III}$ and DMA concentrations were 24 and 50% higher and 12% lower in brown rice than in white rice, respectively.

### 3.4. Grain inorganic As%

Both grain milling and treatment affected grain inorganic As% (percentage of inorganic As relative to the sum of all As species present in

**Table 2**
Soil moisture (soil volumetric water content, VWC, and water potential, WP, at 0-15 cm soil depth, and perched water table, PWT) immediately before reflooding, and soil drying duration. Soil VWC and WP were monitored in one CF plot for comparison.

| Treatment | | VWC | WP | WP mean | PWT[b] | Duration[c] |
|---|---|---|---|---|---|---|
| Severity | Timing | % | kPa | kPa | cm | days |
| CF | | 48 | −9 | - | - | - |
| LS | Panicle Initiation | 50 (1)[a] a | 0 (0) | −1 C | −917.4 (3) | 3 |
| | Booting | 48 (0.4) a | −2 (2) | | −913.0 (3) | 1 |
| | Heading | 47 (0.5) a | 0 (0) | | −919.8 (2) | 2 |
| MS | Panicle Initiation | 33 (1) b | −57 (5) | −71 B | - | 10 |
| | Booting | 38 (1) a | −69 (13) | | - | 8 |
| | Heading | 37 (2) ab | −87 (3) | | - | 11 |
| HS | Panicle Initiation | 33 (1) a | −173 (26) | −154 A | - | 14 |
| | Booting | 35 (1) a | −163 (28) | | - | 12 |
| | Heading | 31 (2) a | −127 (13) | | - | 15 |
| ANOVA | Timing | ns | ns | - | ns | - |
| | Severity | *** | *** | - | - | - |
| | Timing:Severity | * | ns | - | - | - |

Abbreviations: CF=continuously flooded, LS = low severity; MS = medium severity; HS = high severity; *** = $p < 0.001$; * = $p < 0.05$; ns=not significant.

[a] Numbers in parenthesis are standard error of means. Different uppercase letters in a column indicate significant differences ($p < 0.05$). Different lowercase letters within each severity indicate significant differences ($p < 0.05$).

[b] Negative PWT indicates that it is below the soil surface.

[c] The duration of a drying period was the number of days from when the PWT was at the soil surface to when the soil was reflooded.

grain), although there was not an interaction between them (Table 3). White rice (63%) had lower grain inorganic As% than brown rice (75%). The effect of treatment is represented by a set of orthogonal contrasts. The purpose of this analysis was to test the hypothesis that decreases in grain total As concentration caused by soil drying are accompanied by changes in grain As speciation. Since drying the soil to LS had no effect on grain As concentration (Fig. 1), all LS treatments were excluded from this analysis. The estimates obtained from the set of contrasts indicate that, in brown and white rice, grain inorganic As %:1) is higher in the MS and HS treatments compared to CF (on average,

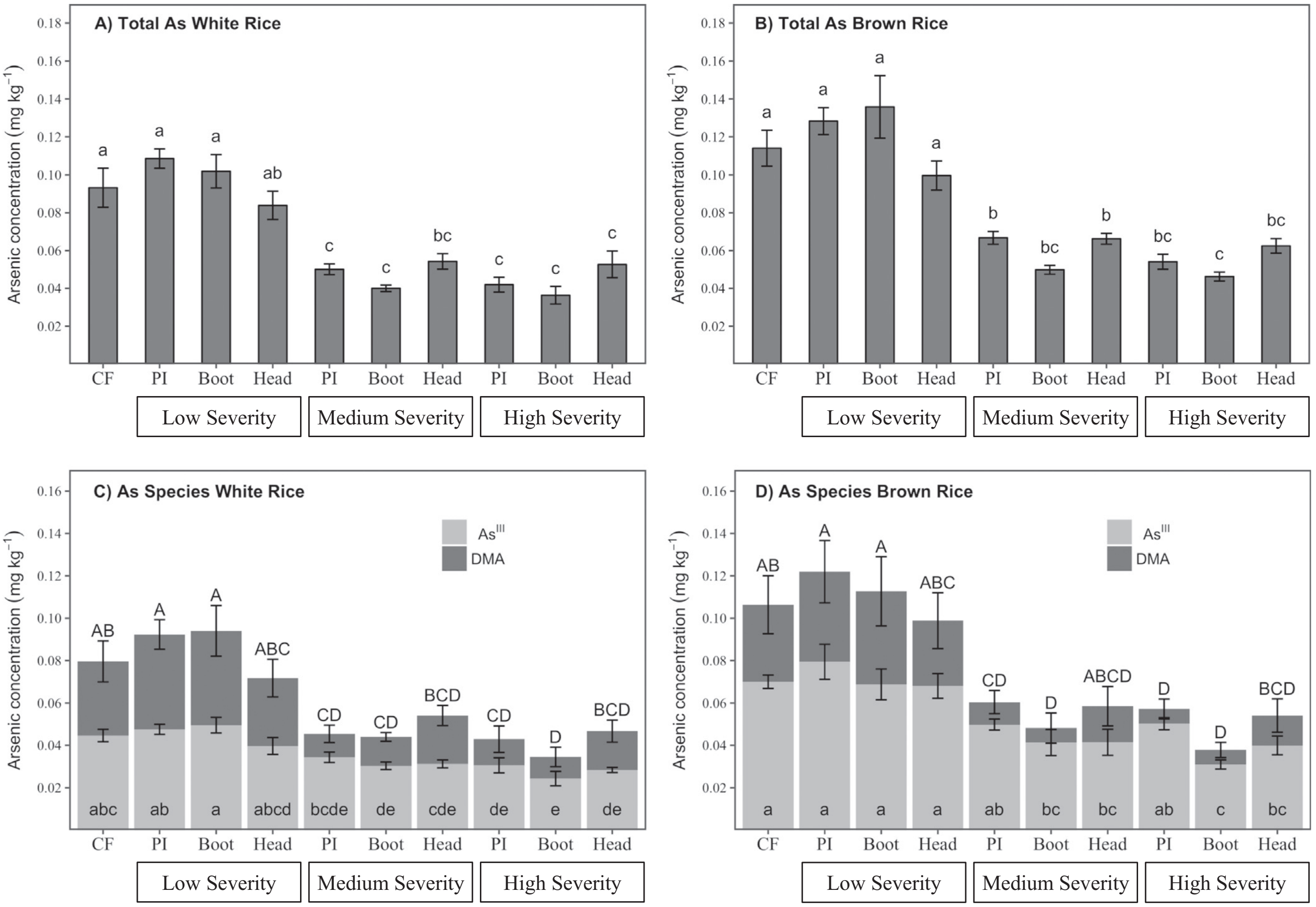

**Fig. 1.** Total As and As species concentration in brown and white rice. $As^V$ and MMA were not detected in any of the samples. Error bars represent standard error of means. Different lowercase or uppercase letters indicate significant ($p < 0.05$) differences between treatments. Abbreviations: CF = continuously flooded, PI = panicle initiation, Boot = booting, Head = heading.

**Table 3**
Percentage of inorganic arsenic relative to all arsenic species present in grain (inorganic As%) in white and brown rice. The concentration of inorganic arsenic (inorganic As, $mg\ kg^{-1}$) is represented for comparison (replicated data from Figure 1). The treatment effect excluded all low severity treatments, since none of them affected grain As concentration.

| Grain inorganic As% (relative to the sum of all As species in grain) | | | | |
|---|---|---|---|---|
| | | Treatment | White rice | Brown rice |
| 1[a] | CF | | 57 (5)[b] | 68 (6) |
| 2 | LS | Panicle Initiation | 52 (3) | 65 (5) |
| 3 | | Booting | 54 (5) | 61 (7) |
| 4 | | Heading | 56 (4) | 69 (6) |
| 5 | MS | Panicle Initiation | 75 (4) | 83 (3) |
| 6 | | Booting | 69 (2) | 85 (3) |
| 7 | | Heading | 58 (3) | 70 (6) |
| 8 | HS | Panicle Initiation | 73 (3) | 88 (2) |
| 9 | | Booting | 70 (1) | 82 (3) |
| 10 | | Heading | 62 (6) | 74 (7) |

Two-way ANOVA

Treatment $^{***}$
Grain milling $^{***}$
Treatment:Grain milling $^{ns}$

| Grain milling | Means[c] (%) |
|---|---|
| White rice | 63 b |
| Brown rice | 75 a |

| Treatment | Orthogonal contrast estimate[d] |
|---|---|
| CF vs. (MS+HS)<br>(1) vs. (3+4+6+7+9+10) | (-) $^{**}$ |
| MS vs. HS<br>(3+6+9) vs. (4+7+10) | ns |
| Panicle Initiation vs. Booting<br>(3+4) vs. (6+7) | ns |
| (Panicle Initiation+Booting) vs. Heading<br>(3+4+6+7) vs. (9+10) | (+) $^{***}$ |
| Interaction (Panicle Initiation vs. Booting)<br>(3+7) vs. (4+6) | ns |
| Interaction (Panicle Initiation +Booting vs. Heading)<br>(3+6+10) vs. (4+7+9) | ns |

Abbreviations: CF = continuously flooded, LS = low severity, MS = medium severity, HS = high severity, $^{*}$ $p< 0.05$, $^{**}$ $p< 0.01$, $^{***}$ $p< 0.001$, ns = not significant.

[a] Numbers are for treatment identification referred to in the set of contrasts.

[b] Numbers in parenthesis are standard error of means.

[c] Means were averaged across treatments since there was not a significant ($p < 0.05$) interaction between grain milling and treatment. Different letters indicate significant ($p < 0.05$) differences.

[d] A positive or a negative sign indicates that the first term of the contrast is, respectively, higher or lower than the second term.

68 vs. 57% for white and 80 vs. 68% for brown rice), 2) is higher if the soil is dried during panicle initiation or booting compared to heading (on average, 72 vs. 60% for white and 85 vs. 72% for brown rice), and 3) is not affected by an interaction between timing and severity of soil drying.

### *3.5. Yield and yield components*

There were no differences between treatments with respect to yield, number of tillers per $m^2$, percentage of unproductive tillers, and 1000-grain weight. However, the harvest index was higher in CF (53%) than when the soil was dried to HS at booting (47%) (Table S2, Appendix A).

## 4. Discussion

Our results indicate that the severity of soil drying plays an important role in mitigating the accumulation of As in rice grain. In contrast to the more severe MS and HS treatments, drying the soil to LS (severity used in the “Safe-AWD”) had no effect on grain total As concentration or As speciation, independent of timing (Fig. 1). This was likely because in the LS treatments the soil moisture never dropped below saturation (i.e., soil WP was ~0 kPa at the end of the drying period) (Table 2), thus, the soil was not aerated sufficiently to alter As soil bioavailability. In contrast, in soils of the same textural class as the one in this study (i.e., clay), others have reported a decrease in grain As concentration when the soil was dried to LS multiple times (Islam et al., 2017; Norton et al., 2017b). This would suggest that the different results observed by Islam et al. (2017) and Norton et al. (2017b) were caused by the higher number of soil drying periods imposed in their studies. However, another study conducted in an adjacent field reported that when two severe (i.e., comparable to MS and HS here) drying periods were imposed (first at panicle initiation, second at booting), total As concentration in white rice decreased by 56–68%, compared to a CF control (Carrijo et al., 2017); similar reductions were obtained here with a single drying period imposed at panicle initiation (46–55%) or booting (57–61%). This suggests that the number of drying periods imposed in the growing season may not be an important factor influencing grain As concentrations. Differences in soil properties (other than textural class) may explain the discrepancy between the results of our study and the studies of Islam et al. (2017) and Norton et al. (2017b), given that the relation between PWT and soil WP is soil-specific (Lampayan et al., 2015). That said, decreases in grain As concentration reported in the studies of Norton et al. (2017b) and Islam et al. (2017) with LS are generally low (16–35%) compared to what was observed here with the higher severities MS and HS.

While all MS (i.e., soil WP of −71 kPa after 10 days drying) and HS (i.e., soil WP of −154 kPa after 14 days drying) treatments decreased total grain As concentration compared to the CF control, this did not always translate into a decrease in $As^{III}$ and DMA concentrations (Fig. 1C and D), because grain As speciation varied with the timing and severity of soil drying (Table 3). Higher grain inorganic As% was observed when severe drying periods (MS and HS) were imposed compared to CF, and when the drying period was imposed earlier (i.e., panicle initiation or booting) rather than later (i.e., heading) in the cropping season. As a result, there were soil drying treatments (e.g., MS-panicle initiation) that were effective in decreasing total As, but not $As^{III}$ concentration. This is important given that inorganic As is the most toxic form of As and the primary focus of public health regulations (Signes-Pastor et al., 2016). Based on these findings, reports on the effect of soil drying on grain total As (e.g., Carrijo et al., 2018, and LaHue et al., 2016) likely overestimate the health benefits of soil drying in reducing grain As. Further, these results indicate that there is not a fixed conversion factor from total As to inorganic As concentration. For example, for white rice the inorganic As% ranged from 52 to 75% depending on the irrigation treatment (Table 3). If, due to the high cost and complexity of As speciation analysis, regulations targeting inorganic As are made based on an estimated percentage from total As, irrigation practices must be taken into account.

Differences in grain inorganic As% observed between the MS and HS treatments and the CF control (Table 3) may be a result of As transformations in the soil triggered by soil drying. Soil microbes can convert inorganic As into organic As and vice-versa (i.e., methylation and demethylation reactions, respectively) and aerobic conditions favor As demethylation over methylation reactions (Frohne et al., 2011; Meharg and Zhao, 2012; Reid et al., 2017). In addition, there is strong evidence that rice plants cannot methylate As and that methylated As species found in grain originate from the soil (Lomax et al., 2012; Jia et al., 2013; Mishra et al., 2017). These findings suggest that soil drying increases the proportion of inorganic As in the soil (relative to the total As), which is then taken up by the plant and transported to the grain. This explains the higher grain inorganic As% in the MS and HS treatments compared to CF (Table 3), as others have observed (Xu et al., 2008; Somenahally et al., 2011; Das et al., 2016).

The lower grain inorganic As% observed when the soil was dried at heading, compared to panicle initiation and booting (Table 3), may be, in part, explained by differences in As species translocation within the plant. Zheng et al. (2011) found that almost all DMA present in the mature grain was transported to the ovary before flowering while most of the inorganic As was unloaded during grain filling. Their findings suggest that any effort made to decrease DMA uptake after flowering would not translate into a decrease in DMA concentration in grains, although that is not true for $As^{III}$. In agreement, all the MS and HS treatments imposed at heading did not decrease DMA but decreased (with the exception of the MS-heading treatment for white rice) $As^{III}$ grain concentration, compared to the CF control. In a pot study, Arao et al. (2009) also found that aerobic treatment after heading was more effective in decreasing $As^{III}$ than DMA grain concentration, compared to aerobic treatment before heading. It is worth noting that although soil drying at heading may be effective in decreasing grain $As^{III}$, it is likely a risky strategy as rice is very sensitive to water stress during flowering and yields and grain quality may be reduced (Sarvestani et al., 2008; Gunaratne et al., 2011).

Grain As speciation also varied with grain milling (Table 3), with brown rice (75%) having higher grain inorganic As% than white rice (63%). This has been previously observed and is attributed to $As^{III}$ being less mobile in the plant than DMA, which results in $As^{III}$ being less efficiently translocated from the bran to the endosperm of the grain (Sun et al., 2008; Carey et al., 2010; Carey et al., 2011).

While grain As concentration was impacted by soil drying, yield (and yield components, except for harvest index) remained similar to the CF control, independent of the timing or severity of soil drying (Table S2, Appendix A). Based on a meta-analysis, yields are generally reduced when the soil WP at root depth is allowed to drop below −20 kPa (Carrijo et al., 2017). However, in this study in both the MS and HS treatments the soil WP dropped below −20 kPa (on average, −71 kPa and −154 kPa for the MS and HS treatments, respectively) and yet yields were not reduced. In a study we conducted in an adjacent field, similar yields were observed between severe soil drying treatments and a CF control, and this was attributed to the availability of water and the presence of roots at deeper soil layers (Carrijo et al., 2018). This suggests that for at least some conditions such as those in this study, yields can be maintained while at the same time As grain concentrations can be reduced.

## 5. Conclusions

This field study indicates that a single soil drying event during the season has the potential to mitigate As accumulation in rice grains. However, the severity and the timing of soil drying will determine if, and to what extent, this potential can be realized. Grain As concentration was not affected unless the soil was dried to achieve negative soil water potentials; in this study, this meant that the soil had to be dried further than it is for “Safe-AWD (alternate wetting and drying)” (i.e., perched water table at 15 cm below the soil surface). In white and brown rice, the lowest grain As concentrations for all species detected were observed when the soil was severely dried [i.e., soil (0–15 cm) water potential of −154 kPa] at the booting stage. Importantly, the timing and the severity of soil drying affected grain As speciation, which resulted in some soil drying treatments being effective at decreasing total As but not inorganic As concentration. Overall, in white and brown rice, the soil had to be dried severely and/or later in the season (at booting or heading instead of at panicle initiation) to decrease inorganic As concentration in grain, compared to a continuously flooded control. This effect of irrigation management on grain As speciation needs to be considered if regulations targeting grain inorganic As are based on an estimated inorganic As percentage from measured total As concentrations.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.08.216.

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