Agriculture, Ecosystems and Environment 272 (2019) 188–198

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Impact of Alternate Wetting and Drying Irrigation on Arsenic Uptake and Speciation in Flooded Rice Systems

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ARTICLE INFO

Keywords:
arsenic
rice
alternate wetting and drying
speciation

ABSTRACT

Alternate wetting and drying (AWD) irrigation can be used to promote oxic soil conditions and decrease arsenic (As) mobility and uptake into rice plants. However, scant information is available quantifying plant As speciation and uptake at the field scale for AWD with different soil drying severities. It is hypothesized that as the severity of soil drying increases, plant uptake and subsequent accumulation of both inorganic and organic As in the grain will decrease. However, since AWD can increase cadmium (Cd) bioavailability, Cd concentrations in rice grains should be evaluated concomitant to As. In this two-year field study, As and Cd uptake were examined, with routine plant and water sampling during the growing seasons, under three AWD practices varying in soil drying severity (from most to least severe: AWD25: drying to 25% volumetric water content at the root zone; AWD35: to 35%; AWDS: Safe AWD, drying to perched water table 15 cm below the soil surface), compared to a continuous flooding (CF) control. Arsenic speciation was also analyzed in grain and vegetative tissues. AWD25 and AWD35 decreased As accumulation in roots and straws by a similar amount compared to CF, leading to a 41-68% decrease in grain total As concentration. Speciation analysis revealed that AWD25 and AWD35 decreased grain concentration of organic As by 70-100% and inorganic As by 14-61% compared to CF. In contrast, AWDS did not decrease As uptake by rice compared to CF. Grain Cd levels were 6.5 μg kg^{-1} in CF, 16.6 μg kg^{-1} in AWD35, and 27.4 μg kg^{-1} in AWD25, suggesting AWD35 could serve as a mitigation option for As, while minimizing Cd accumulation.

1. Introduction

Inorganic arsenic (*i*As) is a non-threshold, Group 1 carcinogen with a linear dose-response for chronic low level exposure (Smith et al., 2002; Ng, 2005). Intake of *i*As in rice is a significant risk factor for cancer in populations for whom rice is a staple food, especially since rice is highly efficient in assimilating arsenic (As) compared with other cereal crops (e.g., wheat or barley) (Das et al., 2004; Mondal and Polya, 2008). Although rice cultivation in some parts of the world has received a great deal of attention due to high levels of *i*As in rice (> 0.2 mg kg^{-1}) as a public health concern (e.g. Williams et al., 2006), increased scrutiny on *i*As levels for particular at risk populations is occurring. For example, the US Food and Drug Administration (FDA) has proposed an action level of 100 μg kg^{-1} of *i*As for rice grains used in infant food products (FDA, 2016). Thus, there is a need to develop soil management strategies for minimizing *i*As accumulation in rice grains for geographic regions with both high and moderate As levels.

In oxic soils, arsenate (As^{V}) predominates and is strongly adsorbed to soil minerals such as iron (oxyhydr)oxides, limiting its movement to soil solution (Onken and Hossner, 1996; Meharg, 2004; Williams et al., 2007; Yamaguchi et al., 2014). In contrast, submerged soils in rice paddy fields exist under anoxic conditions, leading to the increased prevalence of reduced As (As^{III}: arsenite), which is more soluble and mobile than As^{V}. Arsenic is also subjected to methylation reactions in paddy soils and two organic species are commonly reported: monomethylarsonic acid (MMA) and dimethylarsenic acid (DMA) (Masscheleyn et al., 1991; Marin et al., 1992; Zavala et al., 2008; Norton et al., 2009). Arsenic bioavailability and toxicity depends on its chemical form (Marin et al., 1992). Swine studies suggest a gut bioavailability of ~90% for *i*As, but of ~30% for DMA with *i*As more toxic than methylated As compounds after assimilation into the blood stream (Cheng et al., 2004; Juhasz et al., 2006). Consequently, not only the

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https://doi.org/10.1016/j.agee.2018.11.009
Received 20 September 2018; Received in revised form 11 November 2018; Accepted 12 November 2018
Available online 01 December 2018

concentration but also the speciation of As must be ascertained when assessing the risk posed by As in the diet (Abedin et al., 2002a; Williams et al., 2006; Zavala et al., 2008).

Multiple studies indicate that accumulation of As in rice grains can markedly decrease (as much as 10- to 15-fold lower) under sustained oxic growing conditions than under continuously flooded (CF), or anoxic conditions (Xu et al., 2008; Li et al., 2009; Hua et al., 2011). Both DMA and *i*As (but to a lesser extent) accumulation can be decreased under oxic as opposed to anoxic growing conditions (Xu et al., 2008; Li et al., 2009; Hu et al., 2015). Due to the disproportional decrease regarding different As species in rice grains, *i*As constitutes a smaller percentage of total As in grain from the anoxic treatment than from the oxic treatment. Nevertheless, the concentration of *i*As in rice under oxic conditions is still lower (1.1 ~ 2.9-fold across different studies) than under anoxic conditions (Xu et al., 2008; Li et al., 2009; Hua et al., 2011; Hu et al., 2015). However, a substantial yield and grain quality decline has also been observed under sustained oxic conditions (Yamaguchi et al., 2014; Hu et al., 2015). Possible reasons for this include the build-up of nematodes, soil pathogens, and increased weed pressure (Li et al., 2009; Yamaguchi et al., 2014). An additional factor to consider is the bioavailability of other potentially harmful metals. In particular, cadmium (Cd) tends to have the opposite response to soil redox conditions than As (Honma et al., 2016). Under continuously oxic cultivation conditions the Cd concentration in rice can increase 30- to 80-fold, compared to anoxic conditions (Arao et al., 2009; Hu et al., 2013a; Hu et al., 2013b; Hu et al., 2015).

Alternate wetting and drying (AWD) is a promising practice which may combine the beneficial aspects of both oxic and anoxic cultivation. Under AWD, flooded soils are intermittently dried during the growing season. This introduces periods of oxic conditions which decreases As^{III} content in soil solution (Price et al., 2013; Rahman et al., 2015). Importantly, high rice yields are achievable if AWD is implemented correctly (Yang et al., 2009; LaHue et al., 2016). Additional benefits from AWD also include decrease in water use and methane emissions (Linquist et al., 2015; Carrijo et al., 2017). Adoption of AWD, albeit limited, is increasing in countries of East Asia, such as Bangladesh, India, Vietnam and China. With the support from national government programs and on-farm participatory adaptive research, adoption of AWD can be achieved on the 20 million hectares of irrigated rice land worldwide that are estimated to suffer from water scarcity by 2025 (Lampayan et al., 2015).

Despite an increasing interest in AWD, insufficient information is available monitoring As uptake into rice using AWD irrigation at the field scale. Hitherto, rice As uptake studies have primarily relied on hydroponic or potted plants under greenhouse conditions which have been criticized for potentially overestimating the amount of As transferred to the rice grain (Geen and Duxbury, 2009). In addition, field scale AWD studies often only report total grain As concentrations and their conclusions may not apply to all As species in rice (Linquist et al., 2015; Carrijo et al., 2018a). Given the lack of field research that quantify As uptake or speciation under AWD, the objectives of this study were to: 1) compare CF with AWD practices (with three different severities of soil drying) with respect to As accumulation in rice at the field scale; 2) analyze As speciation in different plant parts throughout an entire growth season to understand the accumulation of different As species under AWD practices; 3) determine the optimal severity of the soil drying under AWD practices to control the potential food safety risks based on a minimal uptake of both As and Cd from rice.

2. Materials and Methods

2.1. Study site and experimental design

Field trials were conducted in the summer growing seasons of 2015 and 2016 at the Rice Experiment Station (39°27'47"N, 121°43'35"W) in Biggs, California. Soils in the paddy fields are Esquon-Neerdobe Complex (fine, smectitic, thermic, Xeric Epiaquerts and Duraquerts) with texture of 29% sand, 26% silt and 45% clay, pH of 5.3, 34.2 $cmol_c$ kg^{-1} CEC, 1.06% organic carbon and 0.08% total nitrogen (Pittelkow et al., 2012; LaHue et al., 2016). Plots were comprised of 0.3 ha basins which were laid out in a randomized complete block design with three replications. The climate data relevant to this study is described elsewhere (Carrijo et al., 2018a).

In 2015, one control (i.e. CF) and two AWD irrigation treatments with different soil drying severities (AWD35 and AWD25) were evaluated. An additional AWD treatment, safe-AWD (AWDS), was evaluated in 2016. The chosen treatments aimed to represent a wide range of drying severities under which rice yields may or may not be affected. Safe-AWD has been widely adopted in some Asian countries and considered to not limit rice yields (Bouman, 2007; Lampayan et al., 2015), while AWD25 is considered to limit rice yields based on a meta-analysis (Carrijo et al., 2017). In contrast to CF, where flooding was maintained from sowing to maturity, the AWD treatments had two drying periods which occurred between 48 and 81 days after planting (roughly between panicle initiation and 50% heading). During the drying periods, irrigation was interrupted and floodwater subsided to desired soil moisture before being reflooded. AWD35 and AWD25 treatments were reflooded when the soil moisture at rooting depth (0-15 cm) reached 35% (for AWD35) or 25% (for AWD25) volumetric water content. AWDS plots were reflooded when the perched water table reached 15 cm below the soil surface. Details regarding the soil moisture monitoring and other field management practices are reported by Carrijo et al. (2018a).

2.2. Sample collection and preparation

Sampling schedules for 2015 and 2016 are described in Table 1. In each plot, three locations were selected to collect and composite ponded water (if the plot was flooded), soil and rice plants. One liter of water (in high density polyethylene bottle), two kg soil and 15-20 whole plants were obtained from each plot during each sampling event. Irrigation source water (the same for all plots) was also collected at each water sampling event from the main irrigation pipe leading into the experimental fields. Surface soil (0-15 cm) was sampled with an auger and stored in plastic bags. Water and soil were kept at 4 °C after sampling. When sampling rice plants, they were gently uprooted to obtain the entire plant. The exact number of tillers collected each time was only recorded in 2016. Above ground biomass was separated from roots by cutting the plant at 2 cm above soil. Soil adhering to the roots was gently removed in the field before further cleaning in the laboratory. All plastic and glassware used were previously acid washed in 10% nitric acid.

Water samples were filtered through glass microfiber filter paper (Whatman GF/F). An aliquot of filtered water was analyzed for As speciation (described below) within 24 hours. Upon finishing As speciation, water samples were acidified with nitric acid (67-70%, trace metal grade) to a pH below 2.0 for storage at 4 °C. Soil samples from each plot were fully homogenized, subsampled, frozen at −20 °C and

Table 1
Sampling schedule for 2015 and 2016 based on irrigation management.

Sampling schedule for all plots	2015 Date	2016 Date
Between initial flooding and first drying	June 24th	-
1st drying period approaching	July 6th	July 8th
Dry plots for all AWD treatments	July 16th	-
2nd drying period approaching	July 27th	July 25th
Between final flooding and pre-harvest drain	Aug 27th	Aug 9th and Sep 16th
Prior to harvest	Sep 28th	Oct 11th

freeze-dried. Dry soils were ground, passed through a 60-mesh sieve and stored in the dark at room temperature prior to analyses. Plant roots were washed three times with deionized water to remove remaining soil. Water-cleaned roots contained reddish mottles on surfaces, indicating the presence of iron (Fe^{III}) plaque (Fe-plaque) (Otte et al., 1991), which forms following Fe^{II} oxidation on roots surfaces in the rhizosphere (Otte et al., 1991; Lee et al., 2013). Separation procedures of Fe-plaque from root surfaces and its chemical analysis are provided in the Supporting Information. Roots obtained after the plaque separation procedure will hereafter be designated as “roots without plaque”. Aboveground biomass was further separated on the last sampling dates of both years when the rice was ready for harvest into two fractions: rice grain (i.e. paddy rice) and rice straw (leaves and stem). Roots, straw and paddy rice were oven-dried at 65 °C until constant weight and all these dry weights were recorded in 2016. Paddy rice was dehusked to obtain brown rice and white rice was obtained by polishing the bran from brown rice (Linquist et al., 2015). All plant tissues were ground using a stainless-steel mill, passed through a 40-mesh sieve and stored in the dark at room temperature prior to analyses.

2.3. Chemicals

All water used was 18.2 MΩ cm (Barnstead Nanopure). Trace metal grade nitric acid (67-70%), ammonium phosphate dibasic (≥99%), ammonium hydroxide (28-30%) and cadmium chloride were from Fisher Chemical (USA). Arsenite (1001 mg L^{-1}), arsenate (998 mg L^{-1}) and iron (10 mg L^{-1}) stock standards were from Spex Certiprep (USA). DMA (≥98%) and MMA (≥98.5%) were from ChemService (USA). Certified reference materials (1568b and 2709a) were from National Institute of Standards and Technology (NIST). NIST 1568b was used to assess the accuracy of total As concentration and As speciation for rice flour. NIST 2709a was used to assess the accuracy of total As concentration in soil.

2.4. Total As and Cd concentration in soil

Total As concentration in soil was measured through digestion with nitric, sulfuric, and perchloric acids up to 310 °C on a programmed heating block (AIM 500 block digestion system, AI Scientific, Queensland, Australia). The resulting solution containing As was reduced to As^{III} and quantified by vapor generation inductively-coupled plasma emission spectrometer (VG-ICP-AES; Thermo Scientific iCAP 6000, Cambridge, MA) at 194 nm with a detection limit (DL) of 0.4 μg L^{-1} (Tracy et al., 1991). Total Cd concentration in soil was obtained through digestion with nitric acid and hydrogen peroxide in a closed vessel microwave system (CEM MarsXpress, Matthews, SC) (Sah and Miller, 1992). The digestion solution was quantified by ICP-mass spectrometry (MS) with a DL of 0.01 μg L^{-1}.

2.5. Total As and Cd concentration in water and plant tissues

Acidified irrigation or flood water (analyzed directly) and plant tissues (after digestion, described below) were analyzed via ICP-MS 7900 (Agilent Technologies). Instrumental sensitivity was monitored daily and optimized as required. Arsenic (DL 0.01 μg L^{-1}) was monitored at m/z of 75 and selenium (m/z 77, 78 and 82) was also monitored to identify polyatomic $Ar^{40}Cl^{35}$ interferences on m/z 75. No polyatomic interference occurred and corrections were not necessary. Cadmium was measured at m/z of 111 and 114. Ground subsamples of plant tissues were digested by weighing 0.1-0.5 g tissue (depending on the tissue type) into quartz glass digestion tubes and adding 5 mL of 67-70% nitric acid. The mixture was allowed to stand overnight. The samples were then heated at 105 °C on a heating block until there was no brown vapor being emitted and were evaporated to dryness at 120 °C. The residue was reconstituted in 0.28 mol L^{-1} nitric acid to a weight of 10 g. The resulting solution was filtered through a 0.45 μm nylon filter and diluted 5-10 fold before analysis.

2.6. As speciation in water and plant tissues

Arsenic speciation in irrigation or flood water (analyzed directly) and plant tissues (after digestion, described below) were determined by high performance liquid chromatography (HPLC 1200 series, Agilent Technologies) coupled with ICP-MS 7900. Arsenic speciation (elution order: As^{III}, DMA, MMA and As^{V}) were determined using an anion-exchange column (Hamilton PRP-X100, 250 mm × 4.1 mm × 10 μm) with 10 mmol L^{-1} ammonium phosphate dibasic (pH of 8.25 adjusted with ammonium hydroxide) isocratic mobile phase at 1.0 mL min^{-1} connected to ICP-MS nebulizer with analysis as for total As. Chromatogram peaks matched retention times of mixed As species standards and external calibration quantified As species by peak areas (DL 0.1 μg L^{-1}). Plant tissues (0.2-1.0 g depending on the tissue type) were digested with 10 mL 0.28 mol L^{-1} nitric acid in centrifuge tubes at 95 °C for 90 min. The mixture was centrifuged at 5858 g for 15 min and the supernatant was neutralized with the mobile phase and ammonium hydroxide to a target pH of 6.0 to 8.5. The resulting solution was filtered (0.45 μm nylon) prior HPLC-ICP-MS as for water samples. To ensure quality of analysis, one blank, spike and reference material were included for every ten samples (all analyzed in duplicate). A mass balance was performed between the sum of four As species determined by HPLC-ICP-MS and the total As determined by ICP-MS. Method validation is in the Supporting Information.

2.7. Total As and Cd content in plant tissues

In 2016, the total As or Cd content in rice roots and above ground biomass were calculated for different sampling dates and plotted as element accumulation curves. The total element content (in g ha^{-1}) in different parts of rice was calculated using Eq. (1):

$$\text{Content}_{\text{root, ABG}} = \text{Concentration}_{\text{root,ABG}} \times \text{Tiller density} \times \text{Dry weight}_{\text{root, ABG}} \tag{1}$$

where: concentration is the total concentration of the element in mg kg^{-1} (determination method in Section 2.5); tiller density is in tillers m^{-2} at harvest as determined in Carrijo et al. (2018a); dry weight is in kg (determination method in Section 2.2); ABG is the plant aboveground biomass (straw and, if present, paddy rice).

The rate of As and Cd concentration change over the course of the rice growth (time) was also calculated using Eq. (2):

$$\text{Content change rate} = \frac{\text{Content at } t_{n+1} - \text{Content at } t_n}{\Delta t} \tag{2}$$

where: t_n and t_{n+1} represent two sampling time points next to each other; Δt is the period during between the two sampling events.

In this study, it is assumed that the tiller density did not change from the first sampling event to harvest. This assumption may be inaccurate for the first two sampling events as the tiller density may reach maximum after six weeks of sowing; nevertheless, the tiller density remains relatively constant during the late season (~final 60 days) (Miller et al., 1991).

2.8. Statistics

All statistical analyses were performed in JMP (Version 11.1). For all non-repeated measurements (e.g. grain As or Cd concentrations), a linear model including block and treatment as fixed effects was fit separately for each year. An analysis of variance was conducted followed by means separation using Tukey test (at 5% significance). Shapiro-Wilks and Levene’s tests were used to verify the normality and homogeneity of variance with a logarithm transformation performed if needed. For repeated measurements, specifically straw and root As (or Cd) concentrations, ANOVA was first performed using an exploratory model to test for potential interactions of time and treatment ($p < 0.05$). If there was no interaction, simple mean differences of response variables were evaluated. When there was a significant interaction between time and treatment, treatment effects were analyzed separately on each sampling date.

3. Results

3.1. Water

Total As concentrations in ponded water during the two growing seasons ranged from 0.29 to 1.80 ng g^{-1} (Figure S1) with total As concentrations in irrigation source water fluctuating from 0.31 to 2.66 ng g^{-1} (Table S4). The most common form of As in water was *i*As; DMA was only present in ponded water on June 24, 2015 and in irrigation source water on August 27, 2015. In 2016, no DMA was detected over the course of the rice growth period and total As concentrations in both irrigation source water (1.04 ± 0.50 ng g^{-1}) and ponded water (0.77 ± 0.04 ng g^{-1}) were on average lower compared to 2015 (irrigation source water, 2.13 ± 0.21 ng g^{-1}; ponded water, 0.87 ± 0.05 ng g^{-1}). Different total As concentration and As speciation between 2015 and 2016 in water samples may be caused by different irrigation sources (surface plus ground water mixed in 2015 vs. sole surface water in 2016).

Total Cd concentrations in ponded water during 2015 ranged from 2.9 to 101.3 pg g^{-1} and those for 2016 were from 0 (non-detectable) to 59.4 pg g^{-1} (Figure S2). Similar to As concentrations, higher average total Cd concentrations in irrigation source water were observed in 2015 (28.6 ± 13.5 pg g^{-1}) than in 2016 (4.9 ± 1.5 pg g^{-1}) (Table S4).

3.2. Soil

Total As concentration in soil did not significantly change throughout the two growing seasons across different irrigation treatments (sampling time × treatment, $p = 0.8788$) ranging from 3.61 to 4.20 mg kg^{-1} (overall average of 3.85 mg kg^{-1}) in 2015, and from 3.42 to 4.15 mg kg^{-1} (overall average of 3.78 mg kg^{-1}) in 2016. Total Cd concentration in soil, at harvest in 2016, ranged from 0.18 to 0.22 mg kg^{-1}.

3.3. As concentrations in straw and root

There was a significant interaction between time and irrigation treatments ($p < 0.0001$ for both years); therefore, statistical comparisons were only made among treatments within the same sampling date.

At crop maturity in 2015 and 2016, total As concentration of roots under AWD25 and AWD35 were similar and averaged 51% lower than those in the CF (Figure 1&2). However, AWDS roots did not have different total As concentration compared to CF.

At harvest, total As concentration in CF straw was 2.1-fold higher than AWD25 and 1.7-fold higher than AWD35 in 2015 (Fig. 1). In 2016, straw total As concentration from CF at harvest was 2.0-fold higher than AWD25 and 2.4-fold higher than AWD35 (Fig. 2). Similar to root samples, straw total As concentration from AWDS was similar to CF.

Overall, the total As concentration in roots across treatments (for both years) averaged 14.8 mg kg^{-1}, which was 3.9-fold higher than the average bulk soil As concentration and 23-fold higher than the average straw As concentration (0.64 mg kg^{-1}). However, separation of Fe-plaque from the root revealed that 88-95% of the total As was sequestrated on the root surfaces rather than inside the root. Total As concentration of root without Fe-plaque was one order of magnitude lower than that of the root with plaque but was still 1.9 to 3.6-fold higher than that in the corresponding straw (Table 2).

Arsenic speciation showed no organic As (i.e. DMA, MMA) was detected in roots throughout the growing season (Fig. 1 and 2). Inorganic As was also the major As species in straw samples and DMA was only detected at harvest in 2015 and was no more than 3% of the total As (Fig. 1).

3.4. Elemental analysis in root plaque

Iron content in the plaque of roots from rice at maturity in the CF treatment was 2.1-fold higher than AWD25 and 2.2-fold higher than AWD35, but it was similar to AWDS (Table 3). Arsenic and Fe concentrations in plaque were positively correlated ($r^2 = .8766$, $p = 0.0006$; Table 3) while Cd and Fe concentrations in plaque were negatively correlated ($r^2 = .5060$, $p = 0.0479$; Table 3). Total As concentration in Fe-plaque was two orders of magnitude higher than that in the root without plaque (Table 2 and 3). In contrast, total Cd concentration in Fe-plaque was only 2 to 3-fold higher than that in the root without plaque (Table 2 and 3).

3.5. As concentration in grain

Averaged across years, AWD25 decreased total As concentrations in paddy rice by 57%, in brown rice by 54% and in white rice by 63%, compared to CF (Fig. 3). Compared to CF, AWD35 decreased total As concentrations in paddy rice by 52%, in brown rice by 52% and in white rice by 58%. In 2016, AWDS did not decrease total As concentration in grains, irrespective of the grain type, compared to CF.

As^{III}, As^{V} and DMA were present in the paddy rice across treatments and years (Fig. 3). Paddy rice from AWD25 contained 78% less DMA (averaged value of both years) and 40% less *i*As than CF, and that from AWD35 contained 75% less DMA and 32% less *i*As compared to CF. For brown and white rice, As^{III} was the only species detected from AWD25 and AWD35 treatments (in both years), while both DMA and As^{III} were detected under AWDS and CF treatments. As a result, both AWD25 and AWD35 decreased the DMA concentration by 100% in brown and white rice compared to CF in both years. However, the impact of AWD practices on As^{III} concentrations in brown or white rice varied between years. In 2015, AWD25 and AWD35 decreased As^{III} concentration in brown rice by 61% and 55%, respectively, compared to CF, while neither AWD25 nor AWD35 significantly decreased As^{III} concentration in brown rice in 2016 (Table S6). For white rice, AWD25 and AWD35 decreased As^{III} accumulation by 55% and 45%, respectively, compared to CF in 2015; whereas, in 2016, both AWD25 and AWD35 decreased As^{III} by 32% compared to CF. In 2016, AWDS did not decrease any As species in grains, irrespective of the grain type, compared to CF (Table S6).

3.6. Cd concentration in rice

Cadmium concentrations (only quantified in 2016) in all plant parts, at harvest, were not significantly different under CF and AWDS, which had the lowest levels among the treatments (Fig. 4). Compared to CF, AWD25 increased Cd concentrations in root by 136%, in straw by 316%, and in grain by 322% (averaged value of different grain types). Unlike the trends observed for As, AWD35 significantly differed from AWD25, and increased Cd concentrations, compared to CF, in root by 69%, in straw by 164%, and in grain by 158%.

3.7. Changes in As and Cd content in root and aboveground biomass during the season

In 2016, root As content change rates were similar among treatments (average: 0.59 g As ha^{-1} day^{-1}) before the first drying event

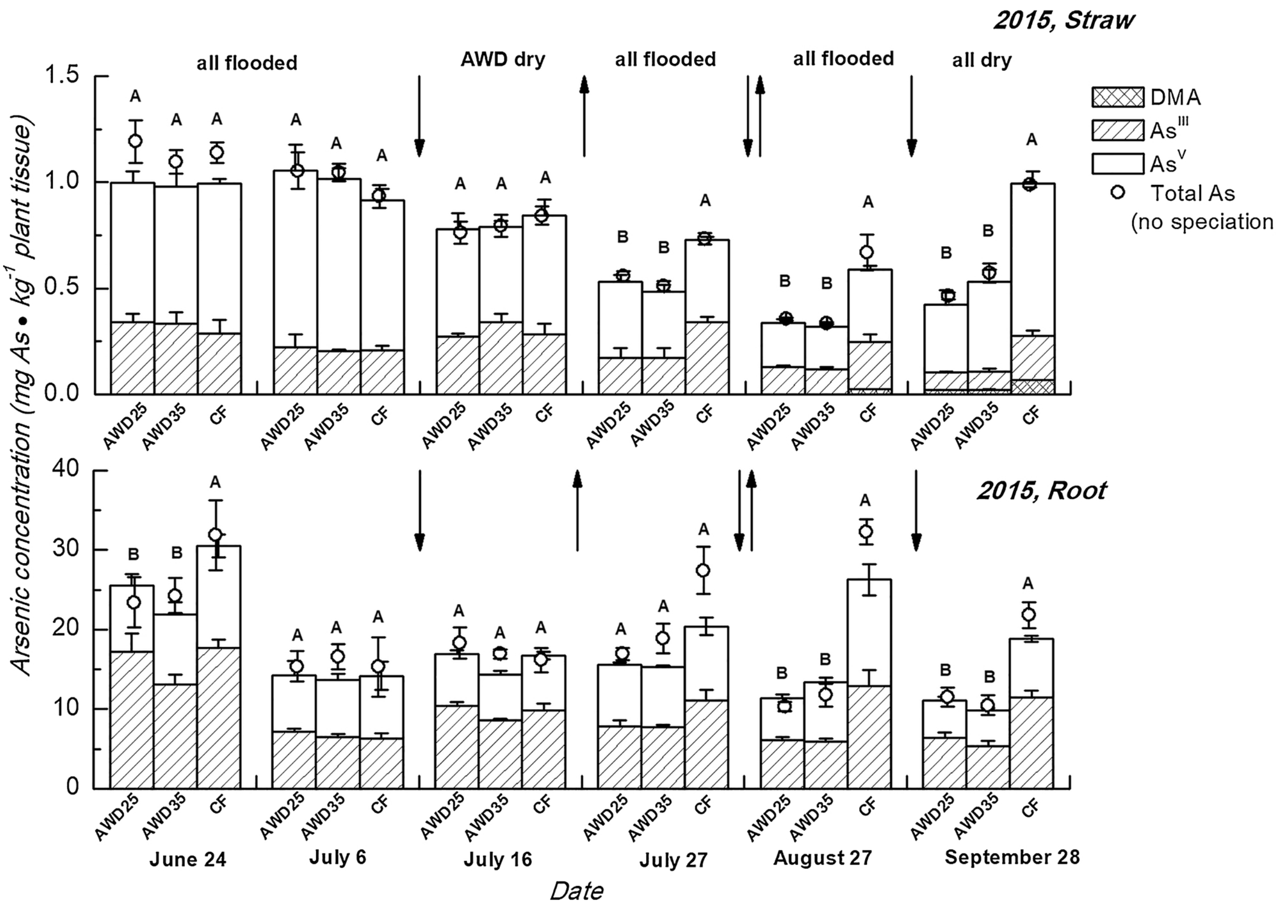

Fig. 1. Concentrations of different arsenic species and total arsenic in straw and root from year 2015. There is a significant interaction between time and irrigation treatments; therefore, statistical comparisons (significance level, $p \leq 0.05$) are made among treatments obtained from the same date regarding the total arsenic concentration. Statistical comparisons regarding the different arsenic species are listed in Table S6. Different letters on top of the bar indicate significant differences between the treatments. ↓ represents a drying event and ↑ represents a reflooding event. All treatments are in triplicates and the values represent average ± standard error. Abbreviations: DMA, dimethylarsinic acid; As^{III}, arsenite; As^{V}, arsenate; AWD25, 25% volumetric water content; AWD35, 35% volumetric water content; CF, continuous flooding.

started in the AWD treatments (Fig. 5). Root As content changed at rates of no more than 0.3 g As ha^{-1} day^{-1} under AWD25 and AWD35 after the first (July 25) and second (August 9) drying events occurred; in contrast, in the CF it increased to 2.8 g As ha^{-1} day^{-1} on July 25 and to 5.7 g As ha^{-1} day^{-1} on August 9. Later in the season (from August 9 to pre-harvest drying), the rate from AWD25 and AWD35 increased to 2.8 g As ha^{-1} day^{-1}, but was still 43% lower than that from the CF. Root As content change rates from AWDS were similar to those from CF throughout the growing season.

Unlike roots, aboveground biomass As content change rates were statistically equal among treatments before and after the two drying-reflooding cycles had occurred (from July 8 through August 9, Fig. 5). However, differences between treatments appeared later in the season (on September 16), with the rate from CF being 2.5-fold higher (averaged value) than that from AWD25 and AWD35.

Under CF, Cd slowly accumulated in root and translocated aboveground from May 24 to September 16 with Cd content change rates fairly constant and below 0.015 g Cd ha^{-1} day^{-1} (Fig. 6). In contrast, Cd content change rate in AWD25 increased with time throughout the drying-reflooding cycles, reaching a maximum of 0.88 g Cd ha^{-1} day^{-1} for root and 0.22 g Cd ha^{-1} day^{-1} for aboveground biomass after the second cycle. Accumulation of Cd in AWD35 were faster than in CF but slower than in AWD25, reaching a maximum rate of 0.43 g Cd ha^{-1} day^{-1} for root and 0.11 g Cd ha^{-1} day^{-1} for aboveground biomass at the end of the second cycle.

4. Discussion

4.1. As and Cd levels in water, soil, and grains

All water samples from this study (Figure S1 and Table S4) are below the United States Environmental Protection Agency permissible limit of 10 ng g^{-1} As (or 5 ng g^{-1} Cd) for drinking water (U.S. EPA, 2017) and Food and Agriculture Organization permissible limit of 100 ng g^{-1} As (or 10 ng g^{-1} Cd) for irrigation water (FAO, 1985). Although there is no globally acceptable permissible As (or Cd) concentration for agricultural soil, 15 mg kg^{-1} has been proposed as the maximum acceptable As concentration in paddy soils and 20 mg kg^{-1} for Cd (Kabata-Pendias, 2001). Irrigation water and soil low in As and Cd led to relatively low grain As and Cd concentrations in this study. Here, the total As concentrations in rice are mostly within global "normal" range of 0.08-0.20 mg kg^{-1} (Zavala and Duxbury, 2008). Nevertheless, *i*As levels greater than 100 μg kg^{-1} were frequently found under CF practice (e.g. *i*As level in brown rice were 104 μg kg^{-1}, Fig. 3), which exceeds the proposed action level for rice used in infant food products (FDA, 2016; Hirsch, 2018). Grain Cd concentrations never exceed the maximum acceptable concentration of 0.4 mg kg^{-1} (Arao et al., 2009).

4.2. Impact of AWD on As content in root and aboveground biomass

Our study revealed that AWD25 and AWD35 began decreasing total

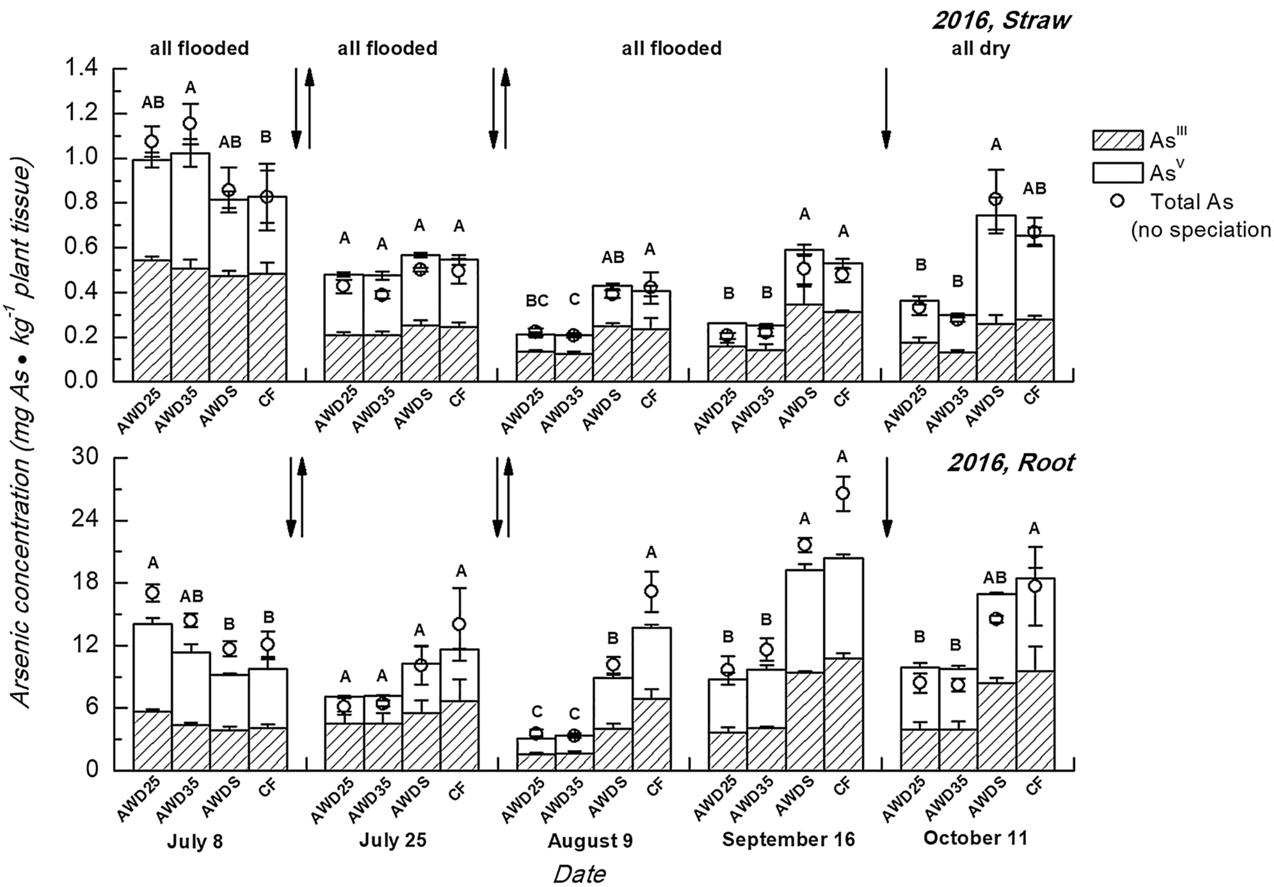

Fig. 2. Concentrations of different arsenic species and total arsenic in straw and root from year 2016. There is a significant interaction between time and irrigation treatments; therefore, statistical comparisons (significance level, $p \leq 0.05$) are made among treatments obtained from the same date regarding the total arsenic concentration. Statistical comparisons regarding the different arsenic species are listed in Table S6. Different letters on top of the bar indicate significant differences between the treatments. ↓ represents a drying event and ↑ represents a reflooding event. All treatments are in triplicates and the values represent average ± standard error. Abbreviations: As^{III}, arsenite; As^{V}, arsenate; AWD25, 25% volumetric water content; AWD35, 35% volumetric water content; AWDS, safe alternate wetting and drying; CF, continuous flooding.

As content in the roots, compared to CF, after the first drying event (i.e. after July 8) in 2016 (Fig. 5). The higher As accumulation in the roots under CF compared to AWD25 and AWD35 suggests that the amount of soluble As in the rhizosphere is higher in the CF treatment. The similarity between CF and AWDS may be because the soil water potential at 0-15 cm soil depth was never below 0 kPa (i.e. soil never dropped below saturation) in AWDS throughout the growing season (Carrijo et al., 2018a) and that oxic conditions did not develop during the drying events. Similar As accumulation in roots between AWD25 and AWD35 indicates that drying the soil to 35% volumetric water content was sufficient for minimizing As uptake and that longer drying times do not further decrease As uptake.

Table 2
Concentration of arsenic and cadmium in root, root without plaque and straw.

Treatment	As^{III} (mg kg^{-1}) Root	Root without plaque	Straw	As^{V} (mg kg^{-1}) Root	Root without plaque	Straw
AWD25	3.9 ± 0.7 (b)	0.35 ± 0.03 (c)	0.18 ± 0.02 (ab)	6.0 ± 0.5 (b)	0.20 ± 0.07 (c)	0.19 ± 0.02 (b)
AWD35	4.0 ± 0.8 (b)	0.34 ± 0.02 (c)	0.13 ± 0.01 (b)	6.0 ± 0.2 (b)	0.24 ± 0.03 (c)	0.17 ± 0.01 (b)
AWDS	8.4 ± 0.4 (ab)	0.82 ± 0.01 (b)	0.26 ± 0.04 (a)	8.5 ± 0.1 (ab)	0.60 ± 0.02 (b)	0.49 ± 0.08 (a)
CF	9.6 ± 2.3 (a)	1.16 ± 0.23 (a)	0.28 ± 0.02 (a)	8.9 ± 1.0 (a)	0.96 ± 0.25 (a)	0.37 ± 0.04 (ab)

Treatment	Total As (mg kg^{-1}) Root	Root without plaque	Straw	Total Cd (mg kg^{-1}) Root	Root without plaque	Straw
AWD25	8.4 ± 0.9 (b)	0.62 ± 0.10 (c)	0.33 ± 0.03 (b)	1.28 ± 0.08 (a)	0.93 ± 0.10 (a)	0.20 ± 0.00 (a)
AWD35	8.2 ± 0.6 (b)	0.70 ± 0.06 (c)	0.28 ± 0.01 (b)	0.92 ± 0.09 (b)	0.66 ± 0.02 (b)	0.13 ± 0.00 (b)
AWDS	14.5 ± 0.3 (ab)	1.69 ± 0.19 (b)	0.82 ± 0.13 (a)	0.64 ± 0.03 (bc)	0.48 ± 0.02 (c)	0.06 ± 0.00 (c)
CF	17.7 ± 3.8 (a)	2.41 ± 0.28 (a)	0.67 ± 0.06 (ab)	0.54 ± 0.10 (c)	0.44 ± 0.02 (c)	0.05 ± 0.00 (c)

All treatments are in triplicates and the values represent average ± standard error. Different letters in parenthesis indicate significant differences ($p \leq 0.05$) between the treatments. Abbreviations: As^{III}, arsenite; As^{V}, arsenate; AWD25, 25% volumetric water content; AWD35, 35% volumetric water content; AWDS, safe alternate wetting and drying; CF, continuous flooding. Samples all from October 11, 2016

Table 3
Elemental analysis of the plaque on the root surface.

Treatment	Mass percent of the plaque on the root (%)	Fe % weight in plaque	As (mg element kg^{-1} plaque)	Cd
AWD25	16.2 ± 1.4 (a)	23.4 ± 7.4 (b)	45.5 ± 7.9 (d)	2.8 ± 0.10 (a)
AWD35	14.6 ± 0.3 (bc)	21.5 ± 2.6 (b)	57.8 ± 5.3 (c)	1.7 ± 0.16 (b)
AWDS	13.5 ± 0.4 (c)	41.0 ± 0.5 (a)	102 ± 1.2 (b)	1.2 ± 0.47 (bc)
CF	15.6 ± 0.9 (a)	48.3 ± 2.4 (a)	110 ± 6.0 (a)	0.9 ± 0.02 (c)

All treatments are in triplicates and the values represent average ± standard error. Different letters in parenthesis indicate significant differences ($p \le 0.05$) between the treatments. Abbreviations: AWD25, 25% volumetric water content; AWD35, 35% volumetric water content; AWDS, safe alternate wetting and drying; CF, continuous flooding. Samples all from October 11, 2016

After August 5, 2016, all AWD plots were reflooded for 45 days until they were drained in preparation for harvest (Carrijo et al., 2018a). However, the rate of As concentration change during this reflooding period under AWD25 or AWD35 was still 41-44% lower than that under CF (Fig. 5), demonstrating the slow kinetics of As^{V} reduction to As^{III} following flooding, requiring several weeks for complete reduction (Onken and Hossner, 1996). In addition, the ferric ions formed during the oxic conditions can compete with As^{V} as a terminal electron acceptor for (a)biotic reactions, prolonging the reduction process from As^{V} to As^{III} in soil solutions (Masscheleyn et al., 1991; Marin et al., 1993).

The effectiveness of AWD25 and AWD35 in decreasing aboveground biomass As content (compared to CF), however, was not observed until after August 9 in 2016 (Fig. 5). Therefore, As was not effectively transferred above ground before August 5 although root As content from CF and AWDS were higher than that from AWD25 and AWD35 during this period. Studies have shown that As assimilated in root cells are not translocated above ground rapidly because As^{III}, upon its induced exposure in root cells, strongly coordinates with thiol (-SH) rich peptides, such as glutathione and phytochelatins, as a detoxification mechanism, and is then stored in vacuoles (Raab et al., 2007; Norton et al., 2010).

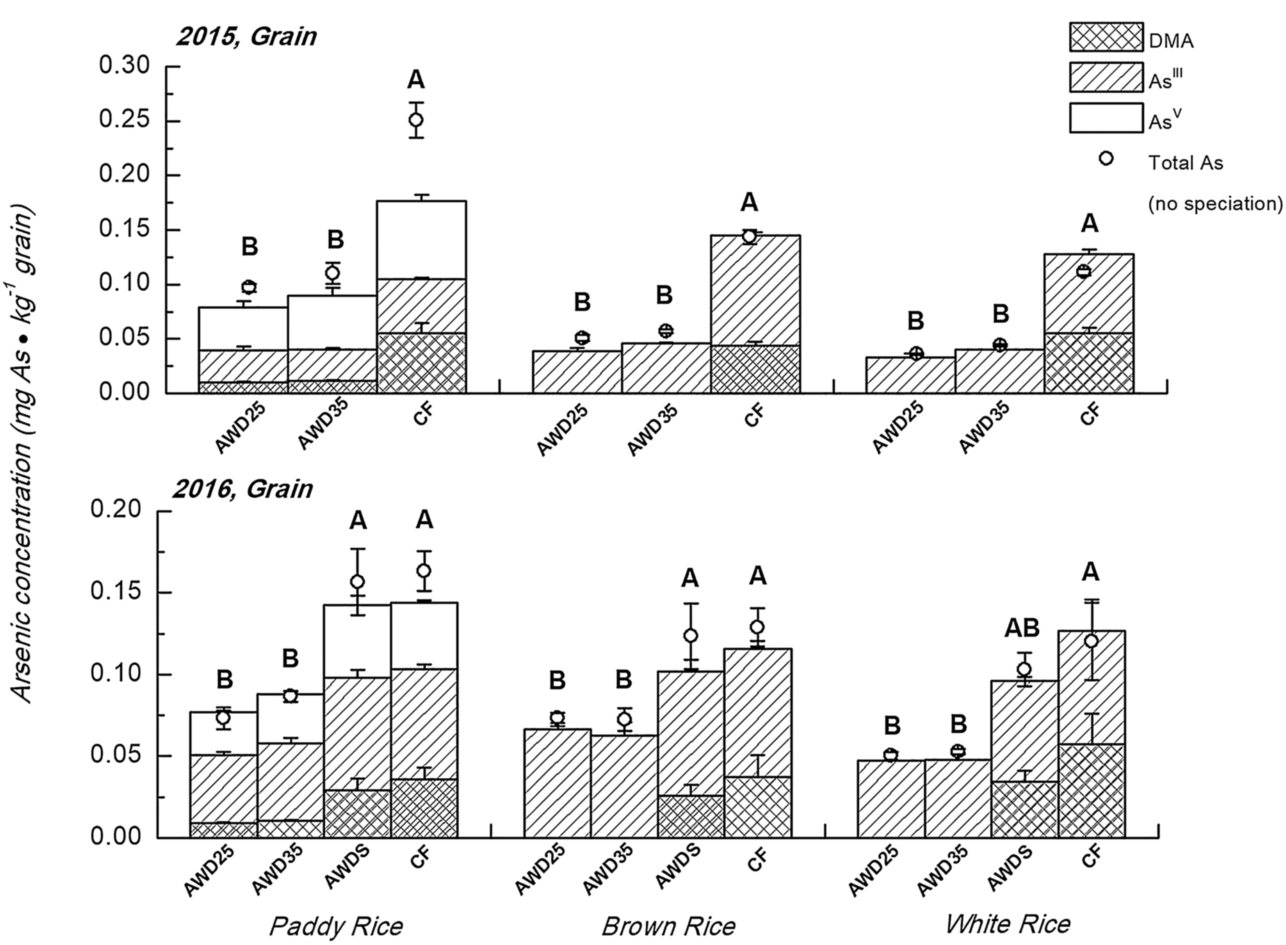

Fig. 3. Concentrations of different arsenic species and total arsenic in rice grains from year 2015 and 2016. Statistical comparisons (significance level, $p \le 0.05$) are made among treatment with the same grain type regarding the total arsenic concentration. Statistical comparisons regarding the different arsenic species are listed in Table S7. Different letters on top of the bar indicate significant differences between the treatments. Abbreviations: DMA, dimethylarsinic acid; As^{III}, arsenite; As^{V}, arsenate; AWD25, 25% volumetric water content; AWD35, 35% volumetric water content; AWDS, safe alternate wetting and drying; CF, continuous flooding.

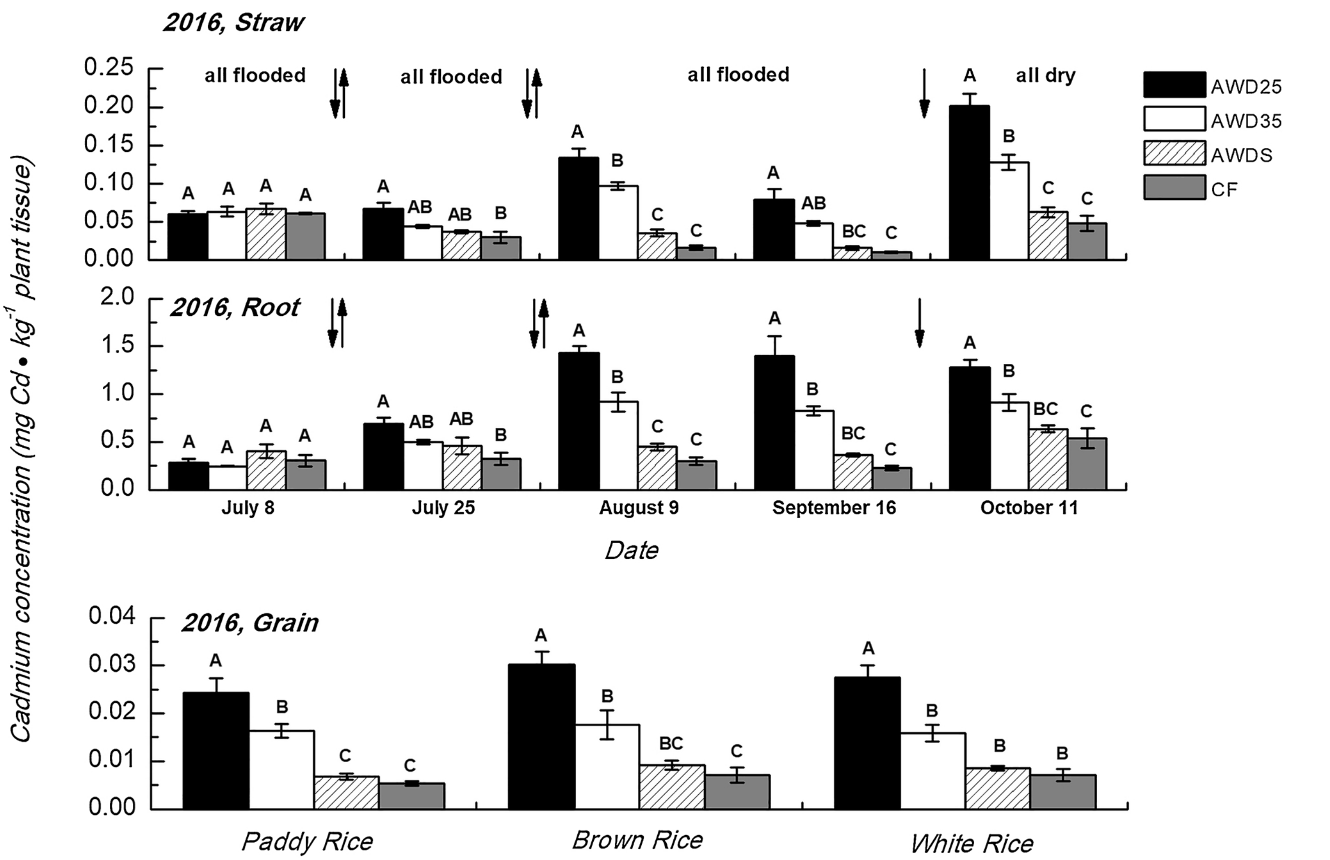

Fig. 4. Concentrations of total cadmium in straw, root and grain from year 2016. There is a significant interaction between time and irrigation treatments; therefore, statistical comparisons (significance level, $p \le 0.05$) are performed among samples obtained from the same date regarding the total cadmium concentration. Different letters on top of the bar indicate significant differences between the treatments. ↓ represents a drying event and ↑ represents a reflooding event. All treatments are in triplicates and the values represent average ± standard error. Abbreviations: AWD25, 25% volumetric water content; AWD35, 35% volumetric water content; AWDS, safe alternate wetting and drying; CF, continuous flooding.

4.3. Impact of AWD on Fe-plaque formation

Formation of Fe-plaque complicates evaluation of As accumulation by rice roots since Fe is also influenced by fluctuating redox conditions (Meharg, 2004; Liu et al., 2010; Somenahally et al., 2011). Our results confirm that anoxic conditions favor Fe-plaque formation compared to oxic conditions (Otte et al., 1991; Liang et al., 2006; Liu et al., 2010; Somenahally et al., 2011), and possible reasons are discussed in the Supporting Information. Our findings also suggest a strong collocation of Fe and As in the root Fe-plaque at plant maturity (Table 3) (Seyfferth et al., 2010). However, the fraction of As entering root cells was not significantly influenced by irrigation practices which confirms that the formation of Fe-plaque may not always function as an effective barrier to As because: 1) different As species have different binding affinities toward Fe-plaque and, 2) saturation in binding As at Fe-plaque surface sites lead to elevated As influx into the roots (Chen et al., 2005; Liu et al., 2005; Seyfferth et al., 2010). More field data, particularly on concentrations of As on root surfaces over time, are needed to clarify whether Fe-plaque is a sink or a source of As for rice when manipulating water regimes (Somenahally et al., 2011).

4.4. Impact of AWD on grain total As and As speciation

Our results indicate that the severity of soil drying plays an important role in not only mitigating grain As accumulation, but also altering grain As speciation. In a recent field-scale study, treatments with one single drying event differing in severity and timing were tested and similar results were obtained (Carrijo et al., 2018b). The authors found that, under the low severity treatment (same severity as AWDS), grain As concentrations (white rice and brown rice) were similar to those under CF. Whereas, the medium severity treatment (same severity as AWD35) and the high severity treatment (similar severity as AWD25) decreased total As by 41-61%. They also found that grain DMA (MMA not detected) concentrations from the high or medium severity treatment were generally lower than those from CF or the low severity treatment.

The concentrations of DMA in rice grain were higher under CF and AWDS, compared to AWD25 and AWD35 (Fig. 3), because microbial methylation in rhizosphere is favored under anoxic conditions (Abedin et al., 2002b), leading to more DMA uptake, compared to oxic conditions, from the pore water into root and subsequent translocation to the grain (Rahman et al., 2008; Somenahally et al., 2011). It is also hypothesized that DMA can be synthesized from *i*As to DMA in plants and the methylation rate of *i*As in rice may accelerate when *i*As loading in grain is increased, which occurs under flooded regimes, to partly alleviate the toxicity of *i*As for plants (Xu et al., 2008). However, recent studies showed 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).

Interestingly, *i*As in the paddy rice consisted of a larger fraction of As^{III} in 2016 (averaged value across treatments: 62% of *i*As as As^{III}) than in 2015 (40% of *i*As as As^{III}). Inconsistent As speciation results between

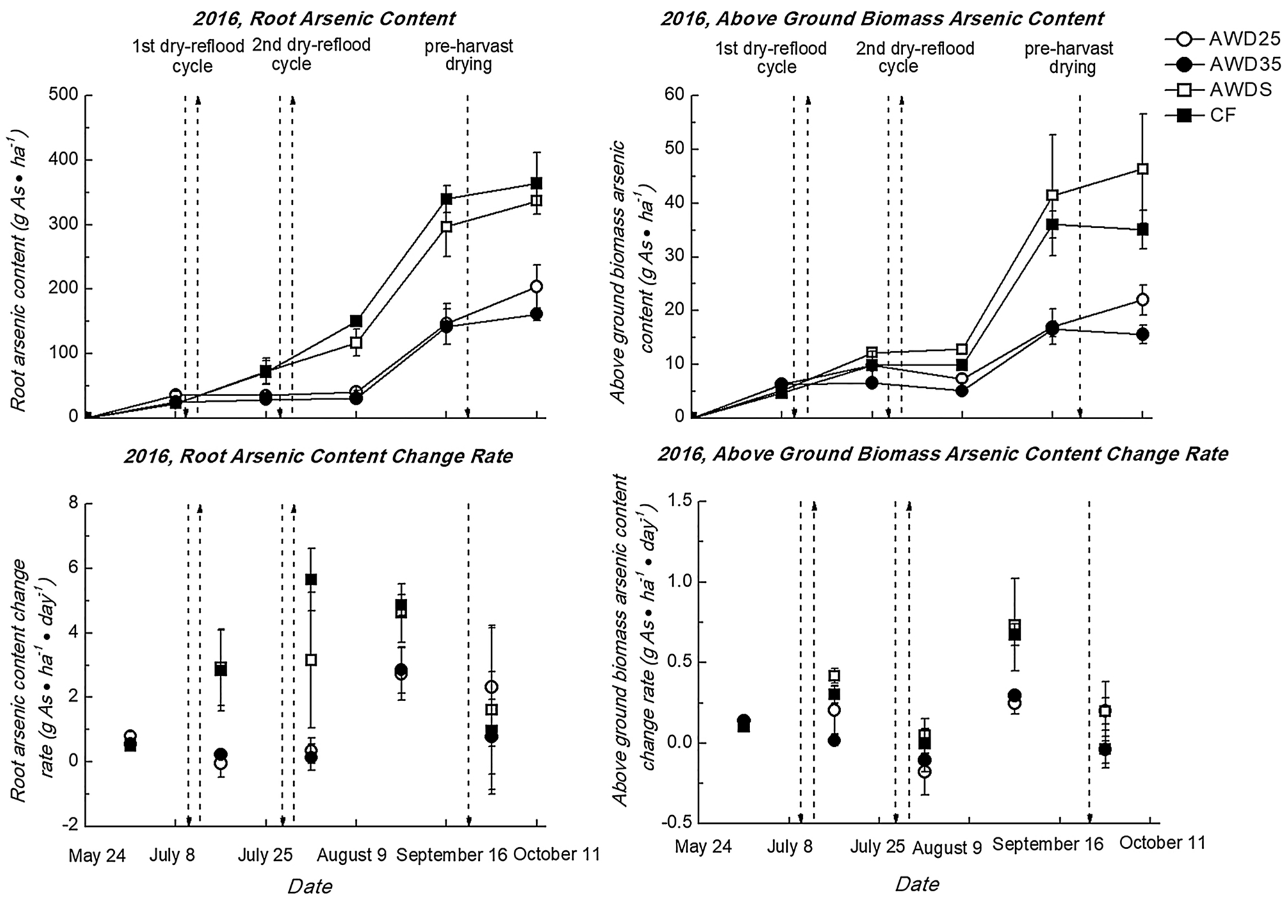

Fig. 5. Root arsenic content, root arsenic content change rate, above ground biomass arsenic content and arsenic content change rate of above ground biomass (samples from year 2016). ↓ represents a drying event and ↑ represents a reflooding event. All treatments are in triplicates and the values represent average ± standard error. Abbreviations: AWD25, 25% volumetric water content; AWD35, 35% volumetric water content; AWDS, safe alternate wetting and drying; CF, continuous flooding.

years may result from different degrees of reduction from the assimilated As^V to As^{III}, driven by endogenous As^V reductases or other non-enzymatic pathways (Xu et al., 2007; Norton et al., 2010).

4.5. Cd response to AWD practice

Oxic conditions can mobilize Cd in the soil, enabling Cd to be assimilated in rice more easily under relatively dry environments than under flooded regime, as opposed to the response of As (Arao et al., 2009; Hu et al., 2013b). Aside from the influence of root uptake and straw remobilization, lower grain Cd concentrations under CF, compared to drier regimes (AWD25 and AWD35), may also be caused by the antagonism between As and Cd in plants since Cd may complex with As; for example, $Cd_3(AsO_4)_2$ (K_{sp} = 2.2 × 10^{-33}), and the co-deposition of As and Cd in maternal tissues decreases the Cd level in grains (Sun et al., 2008).

Under AWD25, which accumulated the most Cd, the aboveground biomass (straw and paddy rice) contained higher level of Cd than the soil (Fig. 4). This suggests that reducing Cd accumulation in rice must be a priority on sites elevated in both As and Cd (Sun et al., 2008; Zhu et al., 2013). A major concern is that Cd has higher mobility than As in rice because Cd can be transported via both phloem and xylem (Tanaka et al., 2007). Aside from the higher mobility, the response of plant Cd concentrations is also more sensitive than As concentrations to water management, since oxidative reactions occur faster than the development of strongly reductive conditions (Arao et al., 2009; Hu et al., 2013a).

4.6. No impact of AWD practices on crop parameters compared to CF

In this study, AWD treatments did not significantly impact yield or yield components (panicle and tiller density, spikelet per panicle and percentage of unfilled grains per panicle) as reported by (Carrijo et al., 2018a). Therefore, the benefits of decreased As accumulation under AWD25 or 35 reported here are achieved without lowering yields.

5. Conclusion

This study provides important field-scale AWD data with variations in severity of drying. Arsenic accumulation into rice tissues were also described over two individual growing seasons. Safe AWD and CF did not differ regarding As and Cd accumulation in rice plants. However, more severe drying regimes (e.g. AWD25 and AWD35) were effective in decreasing the late season (~final 60 days) uptake/transfer of As into the straw, and thus led to lower As concentrations in the grain. These data demonstrates that AWD25 and AWD35 have potential to decrease grain *i*As levels in soils with moderate As concentrations, which may be very important for ensuring that *i*As concentration is below 100 μg kg^{-1} (US FDA proposed action level for infant rice products). This information is critical for refining water management for maximum food safety. Considering potential drawbacks for Cd uptake, AWD35 represented the most beneficial management strategy in this study for minimizing Cd and As uptake simultaneously. A complimentary study is currently underway to examine the impact of AWD on grain nutritional levels, including phosphorous, potassium and zinc, to evaluate other potential consequences of this management strategy.

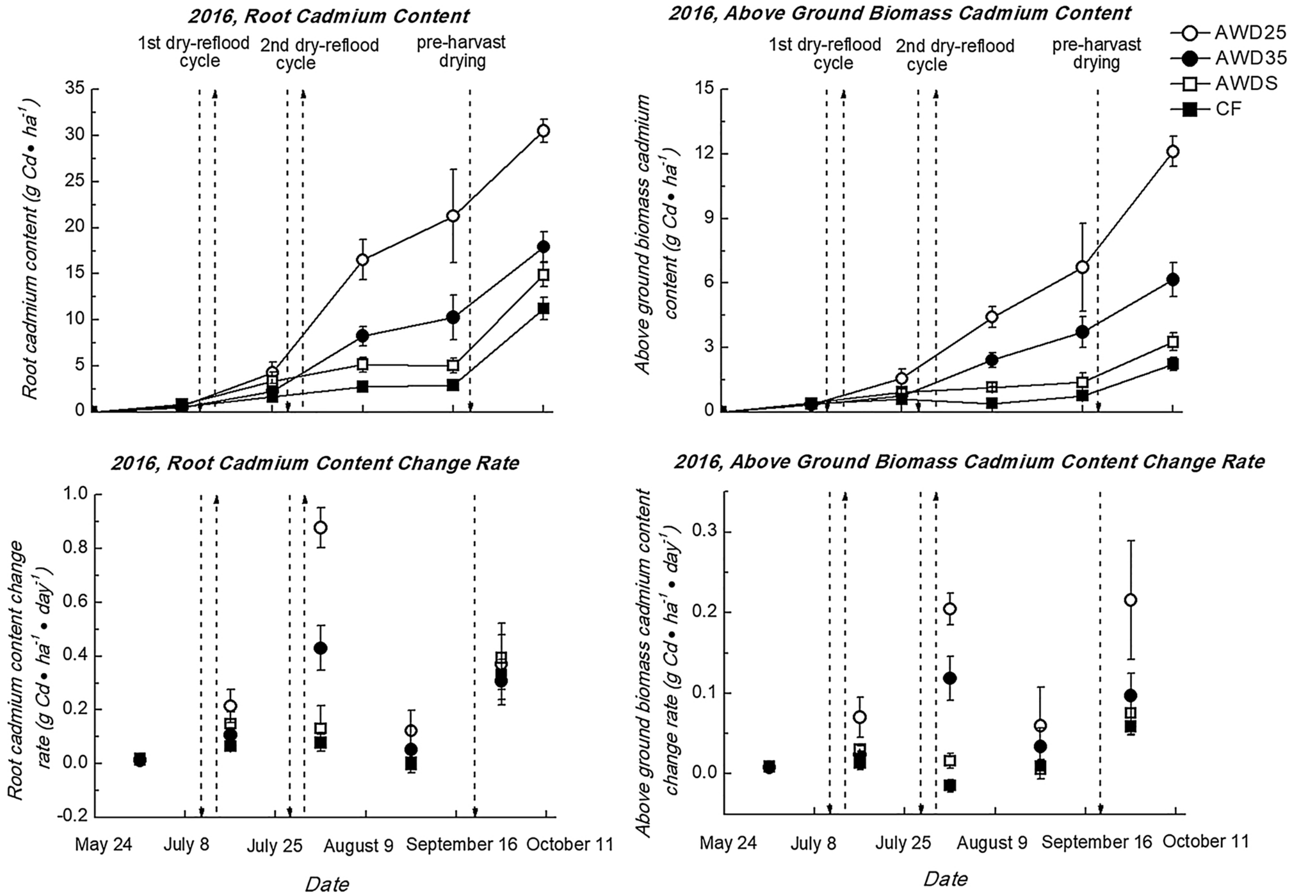

Fig. 6. Root cadmium content, root cadmium content change rate, above ground biomass cadmium content and cadmium content change rate of above ground biomass (samples from year 2016). ↓ represents a drying event and ↑ represents a reflooding event. All treatments are in triplicates and the values represent average ± standard error. Abbreviations: AWD25, 25% volumetric water content; AWD35, 35% volumetric water content; AWDS, safe alternate wetting and drying; CF, continuous flooding.

Acknowledgements

Funding for this work was provided by the California Rice Research Board [grant number: RR1513] and Henry A. Jastro Graduate Research Award. We also thank Andrea Aguilera assisting with plant sample digestions.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agee.2018.11.009.

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