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Impacts of variable soil drying in alternate wetting and drying rice systems on yields, grain arsenic concentration and soil moisture dynamics

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ABSTRACT

Continuously flooded rice systems are a major contributor to global rice production and food security. Allowing the soil to dry periodically during the growing season (such as with alternate wetting and drying irrigation - AWD) has been shown to decrease methane emissions, water usage, and heavy metal accumulation in rice grain. However, the effects of AWD on rice yields are variable and not well understood. A two-year study was established to quantify the impacts of a range of treatments differing in AWD severity (degree of soil drying between flooding events) on yield (as well as factors that may affect yields), soil hydrology in the soil profile, and grain arsenic (As) concentrations relative to a continuously flooded control (CF). Three AWD treatments of increasing severity were imposed between full canopy cover (around 45 days after sowing) and 50% heading: AWD-Safe (field was reflooded when the perched water table reached 15 cm below the soil surface) and AWD35 and AWD25 (field was reflooded when the soil volumetric water content at 0–15 cm depth reached 35% and 25%, respectively). During the drying periods, the 0–15 cm soil layer in the AWD-Safe remained saturated, whereas in AWD35 and AWD25 the soil dried to the desired volumetric water contents. In contrast, soil moisture at 25–35 cm below the soil surface was similar across all treatments. Yield was not reduced in any of the AWD treatments, compared to the CF control. There were no consistent differences in yield components, ^{13}C discrimination, and N dynamics. Results suggest that the availability of water and the presence of roots at the 25–35 cm soil depth during the drying periods ensured that the crop did not suffer drought stress and thus yields were maintained. Grain As concentration in the AWD-Safe treatment was similar to that in the CF control but decreased by 56–68% in AWD35 and AWD25. AWD-Safe is often promoted as a means of practicing AWD without reducing yields; however, in this study this practice did not reduce grain As concentration because the soil did not reach an unsaturated state. These findings demonstrate that knowledge of surface and subsurface hydrology, and the root system are important for understanding the potential of AWD.

1. Introduction

Rice is a staple crop for almost four billion people and the demand for rice is expected to grow through 2025 in response to increasing population (Bouman, 2007). About 75% of global rice production is grown in irrigated lowlands (IRRI, 2017), where the fields are usually continuously flooded throughout the growing season. While continuously flooded rice systems are highly productive, they are associated with a number of issues including high water use (Bouman et al., 2007b), high methane emissions (Linquist et al., 2012), and heavy metal accumulation in the grain [e.g. Zhang et al. (2010) for mercury; Zhao et al. (2010) for arsenic]. Therefore, the development of systems that maintain or increase yields while reducing these negative impacts are important for meeting sustainable intensification goals (Godfray and Garnett, 2014).

Alternate wetting and drying (AWD) has been proposed as an irrigation practice that has the potential to achieve these goals in rice systems. With AWD, fields are subjected to intermittent flooding, where

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irrigation is interrupted and water is allowed to subside via evapotranspiration and percolation until the soil reaches an aerobic state, after which the field is reflooded. Compared to continuously flooded rice systems, AWD has been shown to reduce water use by 23–33% (Carrijo et al., 2017), reduce greenhouse gas emissions (methane plus nitrous oxide) by 45–90% (Linquist et al., 2015a), and reduce methylmercury and total arsenic concentration in rice grain by 38–60% (Rothenberg et al., 2016; Tanner et al., 2018) and 50% (Das et al., 2016), respectively.

While the negative impacts of continuously flooded rice systems can be addressed with the implementation of AWD, in many cases yields are reduced. Based on a meta-analysis, Carrijo et al. (2017) found that the degree of soil drying during the drying events (termed AWD severity) was critical to ensuring that yields were maintained. They reported that compared to continuous flooding, yields were not reduced with mild AWD (soil water potential at root depth > -20 kPa or perched water did not drop below 15 cm from the soil surface) but were reduced on average by 23% with severe AWD (soil water potential at root depth < -20 kPa).

Yield reductions observed using severe AWD may be due to a number of factors. First, it may be water stress. The sensitivity of rice to unsaturated soil conditions can be attributed at least in part to its shallow root system (Parent et al., 2010). Second, AWD may result in increased N losses due to nitrification and denitrification (Pandey et al., 2014), which can lead to reduced plant N uptake. Third, allowing the soil to dry early in the season can promote weed growth (de Vries et al., 2010), leading to increased competition and lower yields. Finally, drying events can increase blast (*Magnaporthe oryzae*) pressure, a major disease of rice which reduces yields (Bidzinski et al., 2016).

There is a high degree of variation across AWD studies with respect to how yields respond to AWD (Carrijo et al., 2017). Lacking in most studies is an understanding of soil hydrology and rooting patterns throughout the rooting depth. Deep roots may be critical for water extraction during the drying periods in AWD (Ludlow and Muchow, 1990) provided that sufficient water is available at depth. Also, at issue is the question of how do AWD benefits (i.e. reductions in methane and heavy metal accumulation) vary with changes in AWD severity. Few studies have evaluated these effects despite the wide range of AWD severities reported in the literature. Therefore, the objective of this study was to establish a range of treatments differing in AWD severity to quantify impacts on yield (as well as factors that may affect yields such as yield components, N uptake, root traits, carbon isotope discrimination), soil hydrology throughout the rooting depth, and grain As concentrations.

2. Material and methods

2.1. Study site characteristics

A two-year field experiment was conducted at the Rice Experiment Station (39°27′47″N, 121°43′35″W) in Biggs, CA during the summers of 2015 and 2016. The soil at the site was 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). The concentrations of total As and Cd in the soil were 3.85 mg kg^{-1} and 0.2 mg kg^{-1} respectively. Total As in the soil was measured through digestion with nitric, sulfuric and perchloric acids up to 310 °C on a programmed heating block. The digested solution was reduced so that all arsenic species were transformed to arsenite and quantified by inductively coupled plasma (ICP) atomic emission at 194 nm using hydride vapor generation (Tracy et al., 1990). Total Cd in soil was obtained through digestion with nitric acid and hydrogen peroxide in a closed vessel microwave system (Sah and Miller, 1992), followed by quantification using ICP-mass spectrometry. The climate at the site is Mediterranean with a mean annual precipitation of 472 mm and average daily temperatures of 15.5 °C (CIMIS, 2017). The total precipitation and average daily temperature during each growing season was 5.2 mm and 22.6 °C in 2015 and 10.1 mm and 21.7 °C in 2016 (CIMIS, 2017), respectively.

Table 1
Summary of management practices in 2015 and 2016.

Crop development and general management practices

	2015		2016	
	Date	DAS	Date	DAS
Fertilization[a]	May 18th	−2	May 23rd	−1
Sowing	May 20th	0	May 24th	0
Initial flood	May 22nd	2	May 26th	2
Canopy cover ≥60%	Jul 1st	42	Jul 11th	48
50% heading	Aug 11th	83	Aug 12th	87
Pre-harvest drain	Sep 7th	110	Sep 19th	118
Harvest	Sep 30th	133	Oct 20th	149

Water management in AWD treatments

	2015			2016		
	Date	DAS	Duration[c]	Date	DAS	Duration
Start of 1st drying period[b]	Jul 7th	48		Jul 12th	49	
AWD-Safe reflooded				Jul 15th	52	3
AWD35 reflooded	Jul 16th	57	9	Jul 19th	56	7
AWD25 reflooded	Jul 19th	60	12	Jul 22nd	59	10
Start of 2nd drying period	Jul 27th	68		Jul 26th	63	
AWD-Safe reflooded				Jul 29th	66	3
AWD35 reflooded	Aug 3rd	75	7	Aug 2nd	70	7
AWD25 reflooded	Aug 9th	81	13	Aug 5th	73	10

[a] Except for N fertilization in the microplots in 2016, which was done immediately prior to the initial flooding of the field.

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

[c] Duration refers to the number of days from the start of a drying period to reflooding. DAS = days after sowing.

2.2. Treatments and experimental design

In 2015, two AWD treatments (AWD35 and AWD25) were compared to a continuously flooded (CF) control irrigation treatment, and an additional AWD treatment (AWD-Safe) was added in 2016. The plots were comprised of 0.3 ha basins, which were precision leveled and had no slope. The basins were separated by levees and drain ditches below the field soil level were constructed between basin levees to prevent lateral seepage between basins. Treatment position within the experimental field was re-randomized in each year. In both years, treatments were arranged in a randomized complete block design with three replications. More detailed information about the treatments and other management practices are reported in Table 1.

In the CF treatment, the field was flooded from sowing to about 3 weeks before harvest. In the AWD treatments two drying periods were imposed where the irrigation was interrupted and the floodwater (i.e. perched water table) was allowed to subside until a certain point before being reflooded. In AWD35 and AWD25 the field was reflooded when the soil moisture at 0–15 cm soil depth reached 35% and 25% volumetric water content, respectively. In AWD-Safe the field was reflooded when the perched water table reached 15 cm below the soil surface (Lampayan et al., 2015). Except for the two drying periods, irrigation, nutrient and pest management in the AWD treatments was similar to the CF control. The first drying period began in all AWD treatments when canopy cover across all plots reached a minimum of 60% (on average 45 days after planting). This was done to suppress weeds that may germinate in unsaturated soil (Rao et al., 2007) and ensure that most of the fertilizer N had been taken up to avoid N losses via coupled nitrification-denitrification (Linquist et al., 2006; LaHue et al., 2016). Canopy cover was monitored weekly (until the start of the AWD cycles) using a line quantum sensor attached to a datalogger (model Li-1400, Li-cor Inc., Lincoln, NE, USA) and was calculated as in Eq. (1):

$$Canopy\ cover\,(\%) = 100 \times \frac{incident\ light - transmitted\ light}{incident\ light} \tag{1}$$

where incident light is the radiation (μmol s^{-1} m^{-2}) measured above the canopy and the transmitted light is the radiation (μmol s^{-1} m^{-2}) measured below the canopy. The second drying period began in all AWD treatments approximately one week after the AWD25 treatment was reflooded (from the first drying period). All AWD treatments were reflooded from the second drying period before 50% heading. In both years, there was no precipitation during the drying periods.

2.3. Field management

In both years, 504 kg ha^{-1} of fertilizer (34-9-5-1.8 N-P-K-S) was banded at a soil depth of 5–7 cm prior to planting. This fertilizer was a blend of mono-ammonium phosphate, urea, ammonium sulfate and muriate of potash, which provided a total of 171, 45, 25 and 2 kg ha^{-1} of N, P_2O_5, K_2O and S, respectively. Seeds of the medium grain variety M-206 were broadcasted onto the dry soil at the rate of 168 kg ha^{-1}, after which the basins were flooded. Herbicides were applied as necessary during the first six weeks of crop development, as is common practice in farmers' fields and were similar for all treatments. About 3 weeks before harvest all plots were drained and allowed to dry in preparation for harvest.

2.4. Soil moisture measurements

In both years, soil volumetric water content at 0–15 cm depth was monitored throughout the season using soil sensors (Decagon Devices 10HS, Inc., Pullman, WA) connected to data loggers. The 10 cm-long sensor probes were installed vertically in the soil, with their centers being positioned at 7.5 cm soil depth. The sensor had a volume of influence of 1 L, which spanned from 0.5 to 14.5 cm soil depth. In 2015, two sensors per plot were used in all plots, whereas in 2016 one sensor per plot was used in all AWD treatments and one sensor was used in a CF plot. In all plots, sensors were installed at least 6 m from basin edges.

Soil water potential, perched water table, and soil gravimetric water content were measured only during the drying periods in 2016. Sensors were installed and measurements were taken at least 6 m from plot edges. Soil water potential was measured at 0–15 cm depth using electrical resistance sensors (Watermark 200SS, Irrometer Co Inc., Riverside, CA). The 8.3 cm-long sensor probes were installed vertically in the soil, with their centers being positioned at 7.5 cm soil depth. One sensor per plot was used in all AWD treatments and one sensor was used in a CF plot. The perched water table was measured using 5 cm diameter polyvinyl chloride tubes perforated with holes approximately 1 cm in diameter and spaced 2 cm apart. The tubes were inserted into the soil after drilling a hole of the exact same diameter and the water level inside the tube was measured with a ruler. All plots were equipped with one 20 cm deep tube (30 cm long with 20 cm below the soil surface), and two of the AWD25 plots contained one 50 cm deep tube (60 cm long with 50 cm below the soil surface). In the AWD35 plots the perched water table eventually dropped below the 20 cm tube depth and thus could not be measured. For these situations, the perched water table in the AWD35 plots was estimated to be the same as what was concomitantly measured in the 50 cm deep tubes in the AWD25 plots or, if a concomitant measurement was not taken, values were interpolated assuming a linear change between the two closest measurements in time (using both measurements from the 20 cm deep tubes and 50 cm deep tubes). Soil gravimetric water content (GWC) was measured immediately before each reflooding event in all AWD treatments, and, by means of comparison, two similar measurements were made in the CF control coinciding with the two drying periods imposed in the AWD treatments. Soil GWC was determined by taking four samples per plot to a depth of 35 cm using a 1.7 cm diameter soil core. Samples were sectioned and pooled into three soil depths (0–15 cm, 15–25 cm and 25–35 cm) and dried at 105 °C until constant weight. Soil gravimetric water content, GWC (%), was calculated as in Eq. (2):

$$GWC = 100 \times \left(\frac{W - D}{D}\right) \tag{2}$$

where: W = sample wet weight (g), D = sample dry weight (g).

2.5. Root measurements

Root measurements were taken in 2016 prior to and after the imposition of the two drying periods, so that all plots were flooded when roots were sampled. Four soil samples per plot were taken to a depth of 35 cm using a 1.7 cm diameter soil core, sectioned and pooled into the three soil depths (0–15 cm, 15–25 cm and 25–35 cm). Samples were washed to remove soil using a 0.1 mm mesh screen and roots were manually separated from organic material. Roots were scanned and analyzed using WinRHIZO software (Regent Instruments Inc., Québec City, QC, Canada) and finally dried at 60 °C and weighed. For each sample, total root length was determined. Root length density (RLD, cm root cm^{-3} soil) and specific root length (SRL, cm mg^{-1}) were calculated for each soil depth as in Eqs. (3) and (4), respectively:

$$RLD = \frac{RL}{4 \times \left(\frac{1.7}{2}\right)^2 \times \pi \times CL} \tag{3}$$

where: RL = total root length present in the sample, given by WinRHIZO (cm), and CL = sample core length (cm).

$$SRL = \frac{RL}{RM} \tag{4}$$

where: RM = oven-dried root mass present in the sample (mg).

2.6. Yield, yield components and $\Delta^{13}C$

When plants were mature, a small plot combine (2.18 m wide) was used to harvest four sample areas of approximately 13 m^2 within each treatment plot. The sample areas were at least 5 m distant from the border of the plot. Grain moisture was measured for each sample, yields were corrected to 14% moisture and the average of the four samples was considered the plot yield. Yield components were obtained by manually harvesting a 1 × 1 m (1 m^2) subplot and subsampling approximately 20% of the fresh biomass. Panicles and tillers in the subsample were counted. The number of spikelets per panicle and percentage of unfilled grains per panicle were obtained from 30 panicles representative of the subsample. Grains were oven-dried at 65 °C, weighed and adjusted to 14% moisture for the estimation of yield. Straw was oven-dried at 65 °C, weighed and the harvest index was obtained as the mass ratio of grain to total aboveground biomass. In 2016 only, grain size was determined by weighing 1000 grains and correcting for 14% moisture.

To determine if the crop experienced drought, carbon isotope composition analysis was performed on the straw dry matter obtained from the subplot used for yield component analysis. Straw samples were ground first in a laboratory mill (Model 4, Thomas Wiley, Philadelphia, PA, USA) fit with a 4 mm screen, and then in a ball mill to pass a 250 μm sieve, after which they were submitted to the Stable Isotope Laboratory at UC Davis for analysis. Carbon isotope discrimination ($\Delta^{13}C$) was calculated according to Farquhar et al. (1989) and assuming a carbon isotope composition of the air of −8‰.

2.7. Field measurements for N uptake determination

Microplots ($2.25\,m^2$) were established to quantify plant N uptake, and its partitioning between fertilizer- and soil-derived N. Immediately prior to the initial flooding of the field, microplots received 180 kg ha^{-1} of N (approximately the same rate as that applied to plots) in the form of ammonium sulfate enriched with 1.1% of ^{15}N. A solution was prepared by diluting ammonium sulfate enriched at 10 atom% into water, and 615 mL was applied uniformly to each microplot using a sprinkling can. Except for N fertilization, management in the microplots was the same as in the plots.

When plants were mature, the above ground biomass of all plants within a $0.5\,m^2$ area in the center of each microplot was manually harvested and a subsample of approximately 50% (fresh weight basis) was oven-dried at 65 °C until constant weight. Grains were separated from straw so that grain and straw yield were recorded separately. Straw was ground first in a laboratory mill (Model 4, Thomas Wiley, Philadelphia, PA, USA) fit with a 4 mm screen, and then in a ball mill to pass a 250 µm sieve, while grains were ground in the ball mill only. In the center of the harvested area, a 1.7 cm diameter soil core was used to take samples (five per microplot) at 0–15 cm and 15–30 cm soil depth. This same procedure was conducted outside the microplot (approximately 10 m away) for the determination of ^{15}N natural abundance. Soil samples were either air-dried (2015) or oven-dried at 60 °C (2016) until constant weight, after which they were homogenized using mortar and pestle and then ground in a ball mill to pass a 250 µm sieve. In addition, bulk density was determined using a 4.5 cm diameter soil core (one per microplot) taken inside the harvested area at the 0–15 cm and 15–30 cm soil depths. Bulk density samples were oven-dried at 105 °C until constant weight. Grain, straw and soil samples, in addition to a sample of the ^{15}N enriched fertilizer solution, were analyzed for N concentration and ^{15}N atom% concentration at the Stable Isotope Laboratory at UC Davis.

^{15}N atom% excess (AE, %) in grain, straw, soil and fertilizer was calculated as in Eq. (5):

$$AE_{grain,straw,soil,fertilizer} = AC_{grain,straw,soil,fertilizer} - AC_{nat} \quad (5)$$

where AC is ^{15}N atom% concentration (%) and AC_{nat} is ^{15}N natural abundance (%), which was determined as the average ^{15}N atom% concentration (%) of all plots soil samples (0–15 and 15–30 cm) collected outside the microplot area.

Total N (TN, kg ha^{-1}) in grain, straw and soil was calculated as in Eqs. (6) and (7):

$$TN_{grain,straw} = N_{grain,straw} \times Y_{grain,straw} \quad (6)$$

$$TN_{soil} = N_{soil} \times BD \times SD \times 10000 \quad (7)$$

where N is N concentration (g g^{-1}), Y is yield (kg ha^{-1}), BD is soil bulk density (kg m^{-3}), and SD is soil layer depth (m).

Fertilizer N recovery (FNR, %) in grain, straw and soil was calculated as in Eq. (8):

$$FNR_{grain,straw,soil} = 100 \times \frac{AE_{grain,straw,soil}}{AE_{fertilizer}} \times \frac{TN_{grain,straw,soil}}{180} \quad (8)$$

Fertilizer use efficiency (FUE, %) was calculated as in Eq. (9):

$$FUE = FNR_{grain} + FNR_{straw} \quad (9)$$

Fertilizer loss (Loss, %) was calculated as in Eq. (10):

$$Loss = 100 - (FNR_{grains} + FNR_{straw} + FNR_{soil}) \quad (10)$$

Plant total N uptake (N_{total}, kg ha^{-1}) was calculated as in Eq. (11):

$$N_{total} = TN_{grain} + TN_{straw} \quad (11)$$

Plant N uptake derived from the fertilizer ($N_{fertilizer}$, kg ha^{-1}) was calculated as in Eq. (12):

$$N_{fertilizer} = \frac{(FNR_{grain} + FNR_{straw}) \times 180}{100} \quad (12)$$

Plant N uptake derived from the soil (N_{soil}, kg ha^{-1}) was calculated as in Eq. (13):

$$N_{soil} = N_{total} - N_{fertilizer} \quad (13)$$

2.8. Grain total As concentration

Rice grains were dehulled, polished, and ball milled to pass a 250 µm sieve. Samples of 0.5 g (two analytical replicates per plot) were digested in glass digestion tubes by adding 5 mL of nitric acid (trace metal grade, 67–70%, Fisher Chemical, USA) 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 M 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 18.2 MΩ cm water (Barnstead Nanopure).

Total As in samples 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. No interferences were observed. Every 10 samples, one blank, one fortified sample, and one certified reference material (1568b, National Institute of Standards and Technology, Gaithersburg, MD, USA) were included as quality control samples. All quality control elements were within the standard quality control criteria, as defined in the FDA Elemental Analysis Manual (FDA, 2012).

2.9. Data analysis

All statistical analyses were performed in R software (R Core Team, 2016). For all measurements with the exception of RLD and SRL, a linear model was fit for each year of experiment including block and treatment as fixed effects. An analysis of variance was conducted followed by means separation using the Fisher Least Significant Difference method. Analyses on RLD and SRL were done by fitting a linear mixed effects model with block, treatment and time (and the interaction between treatment and time) as fixed effects and plot as a random effect, and an analysis of variance was followed by means separation using the Tukey test.

3. Results

3.1. Soil moisture dynamics during the drying periods

The duration of the drying periods in the AWD treatments (i.e. from the day the perched water table was at the soil surface until the field was reflooded) varied from 3 days in AWD-Safe to 13 days in AWD25 (Table 1). From sowing to maturity (pre-harvest drain), volumetric water content (VWC) in the 0–15 cm soil profile in the CF control averaged 47% in 2015 and 50% in 2016 (Fig. 1). In the AWD-Safe treatment, the minimum VWC was 41% and 44% in the first and second drying periods of 2016, respectively. In both years, the minimum VWC in the AWD35 and AWD25 treatments was close to what was targeted and ranged from 30 to 33% in AWD35 and 24 to 28% in AWD25.

Soil water potential (SWP) at 0–15 cm depth never dropped below zero in the CF, as expected (Fig. 2a). Similarly, in the AWD-Safe treatment the SWP never dropped below zero even during the two drying periods. In contrast, negative SWP values were observed in the AWD35 and AWD25 treatments beginning four days after the start of each drying period (i.e. perched water table at the soil surface). The minimum SWP, measured immediately before the first and second

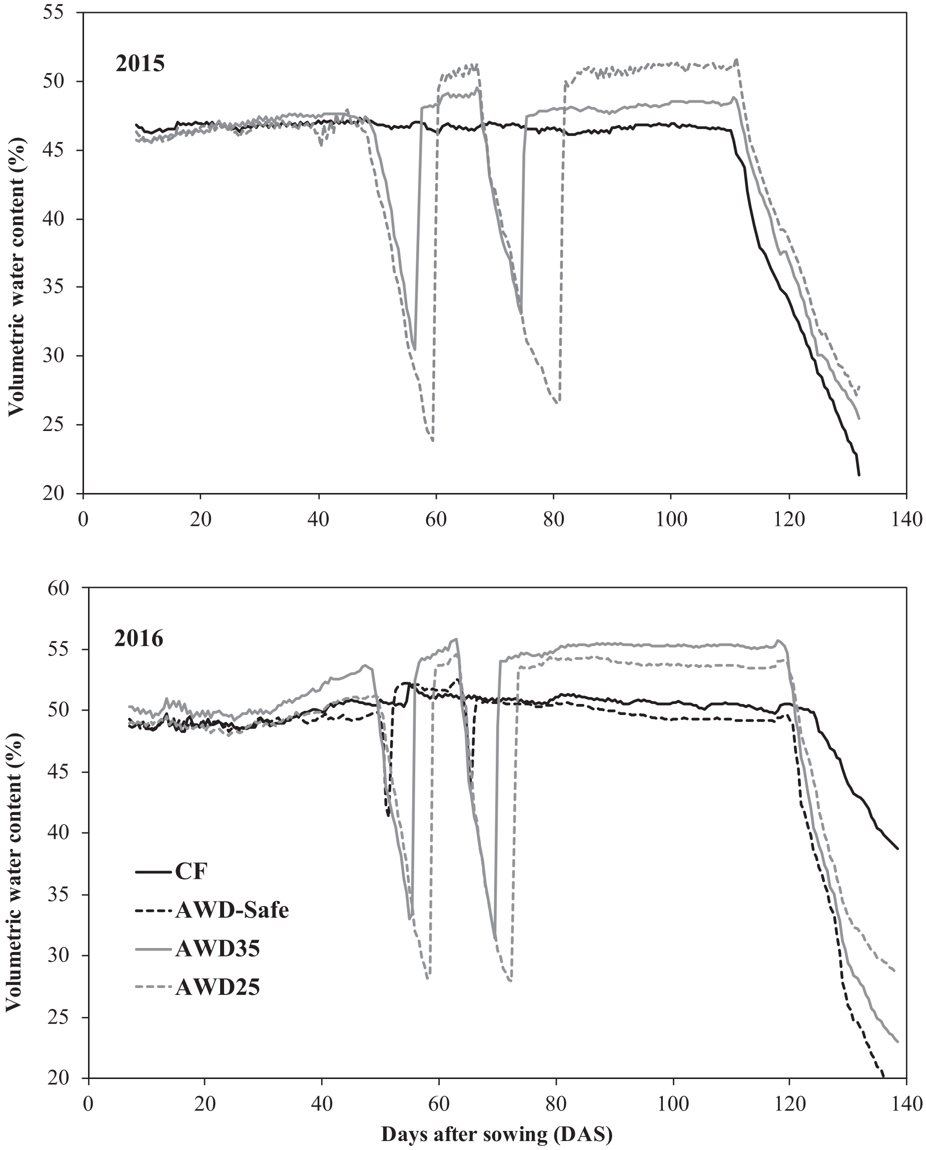

Fig. 1. Volumetric water content measured throughout the season at 0–15 cm depth. Average standard error across all measurements was 0.84%, 0.77%, and 0.85% in CF, AWD35 and AWD25, respectively, in 2015, and 1.1%, 1.4%, and 1.7% in AWD-Safe, AWD35 and AWD25 in 2016.

reflooding events was, respectively, −32 kPa and −35 kPa in AWD35, and −69 kPa and −73 kPa in AWD25.

The perched water table, measured when the drying periods were imposed in the AWD treatments in 2016, averaged 13 cm above the soil in CF (Fig. 2b). The AWD-Safe was reflooded when the perched water table was −19 cm (first drying period) and −20 cm (second drying period), thus slightly lower than the targeted value of −15 cm. The AWD25 was reflooded when the perched water table was −38 cm and −43 cm at the end of the first and second drying periods, respectively. The estimated perched water table in the AWD35 treatment was −31 cm and −34 cm at the end of the first and second drying periods, respectively.

While SWP and VWC were measured only at 0–15 cm, gravimetric water content (GWC) was measured at deeper soil layers immediately before each reflooding event. At 0–15 cm soil depth, GWC followed the same trend as VWC, increasing in the order: AWD25 < AWD35 < AWD-Safe < CF, although the difference between AWD-Safe (44 and 42% at the end of the first and second drying period) and CF (47% at the end of both periods) was not significant (Fig. 3). Differences between the treatments became less evident with increasing soil depth and were not significant at 25–35 cm, with GWC ranging across treatments from 26 to 30% and from 25 to 29% at the end of the first and second period, respectively. In addition, GWC changes with soil depth were different between the treatments. For example, in the CF, AWD-Safe and AWD35 treatments, GWC decreased with soil depth, with the decline being highest in the CF treatment and lowest in the AWD35 treatment. In contrast, GWC increased slightly with soil depth in the AWD25 treatment.

3.2. *Crop parameters*

The AWD treatments did not impact yield in either year compared to the CF control (Table 2). Averaged across treatments, yields were 13.7 t ha^{-1} in 2015 and 11.3 t ha^{-1} in 2016. Similarly, there were no consistent differences in the measured yield components between treatments (Table S1, Supplementary Material). On average, respectively for 2015 and 2016, there were 880 and 559 tillers per m^2, 873 and 553 panicles per m^2, 85 and 91 grains per panicle, 14% and 8% unfilled grains, and a harvest index of 50% and 51%. In 2016, the 1000-grain weight at 14% moisture was 30 g.

Carbon isotope discrimination analysis showed similar Δ^{13}C values across the treatments in both years. Averaging across treatments, Δ^{13}C

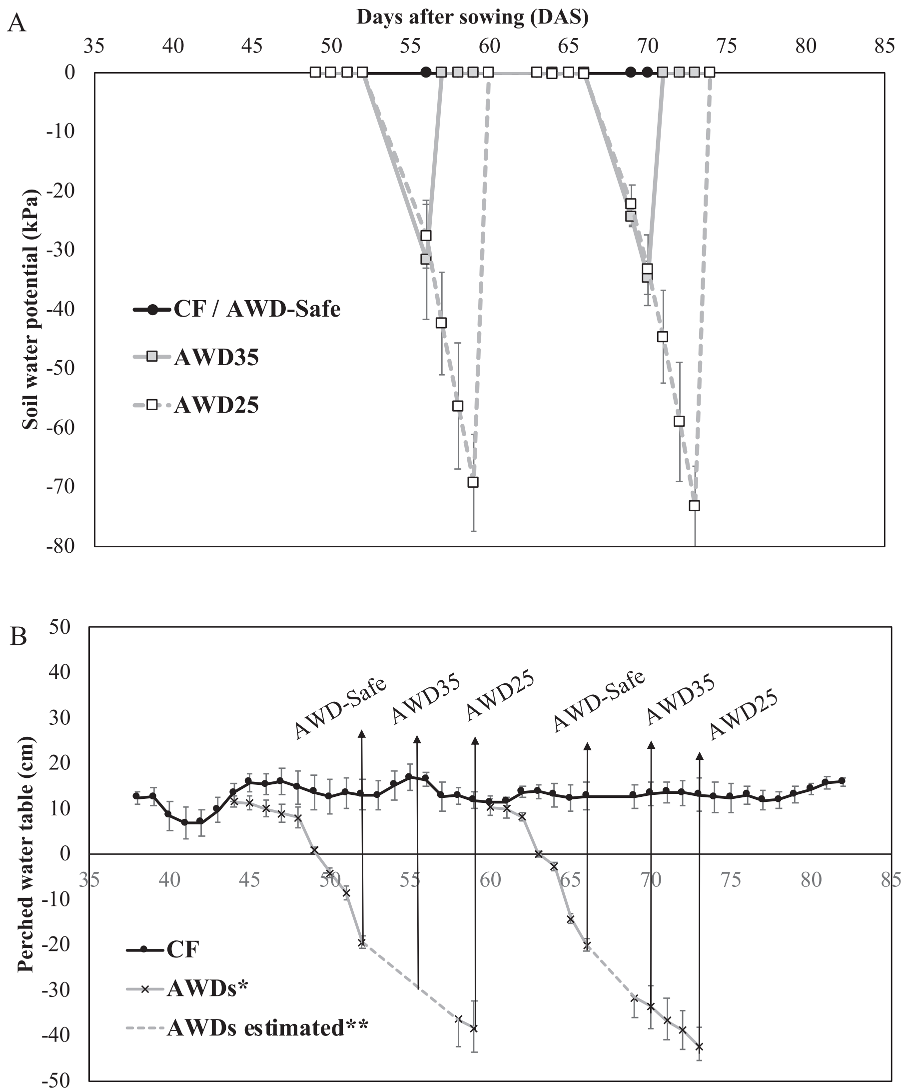

Fig. 2. Soil water potential at 0–15 cm soil depth (A) and perched water table (B) measured throughout the AWD cycles in 2016. Arrows indicate reflooding events. Bars represent standard errors of means.
*AWDs: values above −20 cm are averages of all AWD plots ($n = 9$), measured using the 20 cm deep tubes, and values below −20 cm are averages of two AWD25 plots, measured using the 50 cm deep tubes. **AWDs estimated: values were obtained by interpolation.

values were 20.5‰ (ranging from 20.4 to 20.6‰) in 2015, and 20‰ (ranging from 19.4 to 20.2‰) in 2016 (Table S1, Supplementary Material).

In both years, there were no differences in plant total N uptake between treatments (Table 3). Similarly, in both years, plant N contributions from soil and fertilizer, FUE and the total amount of fertilizer N unaccounted for (Loss) were the same among treatments. Averaged across treatments, N recovery in soil at the 0–15 cm depth was 22% in

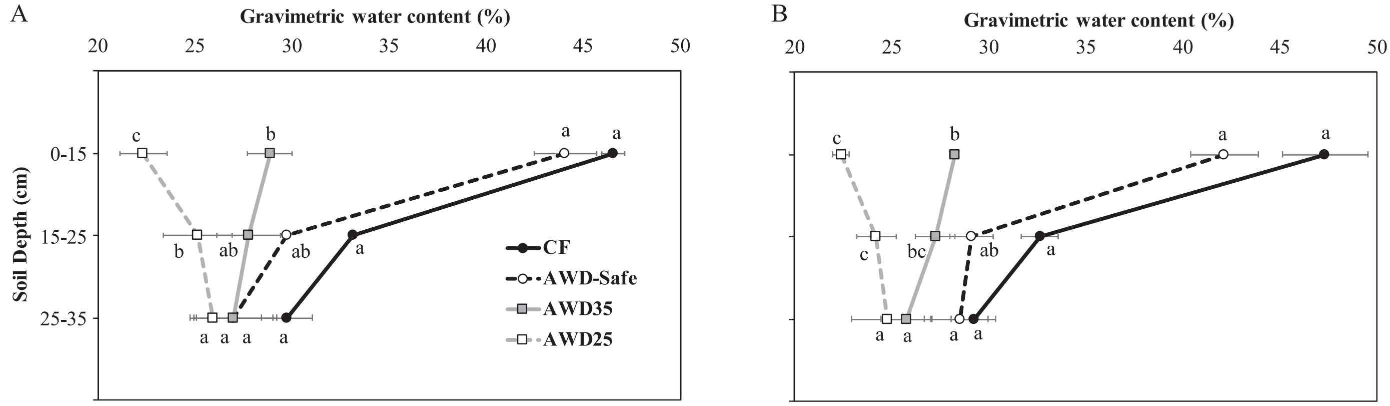

Fig. 3. Soil gravimetric water content at the end of the first (A) and second (B) drying periods in 2016. Measurements in AWD treatments were made immediately prior to reflooding. Measurements in CF were made coinciding with each drying period for comparison. Bars represent standard errors of the means. For each soil depth and drying period, different letters indicate significant differences ($p < 0.05$) between the treatments.

Table 2
Yield (adjusted to 14% moisture) in 2015 and 2016. There was not an AWD-Safe treatment in 2015. Numbers in parenthesis are standard errors. Different letters in a column indicate significant differences ($p < 0.05$) between treatments.

	Yield (t ha^{-1})	
Treatment	2015	2016
CF	13.8 (0.08) a	11.4 (0.08) a
AWD35	13.5 (0.15) a	11.4 (0.09) a
AWD25	13.7 (0.43) a	11.1 (0.28) a
AWD-Safe		11.4 (0.18) a

Table 3
Plant (straw + grain) total N uptake (N_{total}), based on N analysis. Fertilizer-derived N uptake ($N_{fertilizer}$), soil-derived N uptake (N_{soil}), fertilizer use efficiency (FUE) and fertilizer losses (Loss) are based on ^{15}N analysis. Numbers in parenthesis are standard errors of means (n = 3). Within each year, different letters in a column indicate significant differences ($p < 0.05$) between treatments.

Treatment	Year	N_{total} (kg ha^{-1})	$N_{fertilizer}$ (kg ha^{-1})	N_{soil} (kg ha^{-1})	FUE (%)	Loss (%)
CF	2015	157 (11) a	59 (8) a	98 (4) a	33 (5) a	49 (6) a
AWD35		158 (8) a	51 (6) a	107 (5) a	28 (3) a	44 (5) a
AWD25		144 (18) a	42 (4) a	102 (15) a	23 (2) a	48 (6) a
CF	2016	139 (20) a	33 (1.3) a	106 (22) a	18 (0.7) a	41 (3) a
AWD35		124 (4) a	33 (2.7) a	91 (4) a	19 (1.5) a	42 (12) a
AWD25		140 (3) a	34 (0.3) a	106 (2) a	19 (0.2) a	30 (16) a
AWD-Safe		157 (4) a	40 (2.3) a	117 (5) a	22 (1.3) a	33 (9) a

2015 and 43% in 2016, and at the 15–30 cm depth was 3% in 2015 and 1% in 2016 (data not shown).

Root length density (RLD) and specific root length (SRL) were determined before and after the imposition of the two drying periods. There was no difference between treatments in the initial RLD and SRL measurements taken before the drying periods were imposed (Table 4). After the AWD treatments were imposed, the RLD at 0–15 cm and 15–25 cm soil depth was similar in all treatments; however, at the deepest soil layer measured (25–35 cm) RLD was higher in AWD-Safe (1.6 cm cm^{-3}) than in CF and AWD25 (both being 0.8 cm cm^{-3}), while plants in AWD35 had an intermediate RLD (1.4 cm cm^{-3}). Across all treatments, final RLD at 25–35 cm represented only 6% of total RLD, whereas 89% of total RLD was found in the top 15 cm of the soil. The SRL after the imposition of the treatments was not different between the treatments in any of the soil depths measured.

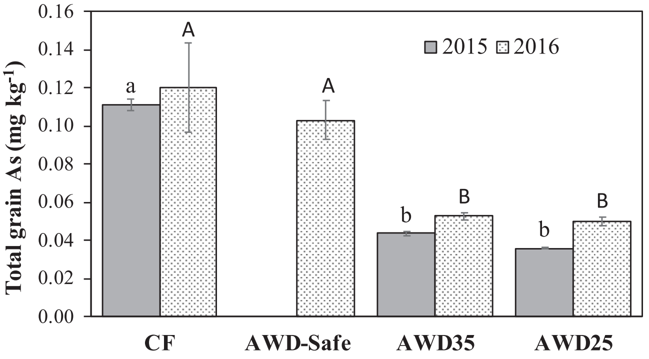

Fig. 4. Grain arsenic concentration in polished rice. Bars represent standard errors of means. Different lower and upper-case letters indicate significant differences ($p < 0.05$) between the treatments in 2015 and 2016, respectively.

3.3. Grain arsenic concentration

In both years, the AWD35 and AWD25 treatments provided similar reductions in grain As concentration (61% and 68% in 2015, 56% and 58% in 2016, respectively) compared to CF (Fig. 4). In contrast, the AWD-Safe treatment had similar grain As concentration as the CF control.

4. Discussion

4.1. Soil moisture dynamics and crop parameters

This study evaluated a range of AWD severities. In the least severe treatment (AWD-Safe) the soil at 0–15 cm depth remained near saturation (i.e. SWP close to 0) and required a drying period of 3 days. In the AWD35 treatment the SWP (0–15 cm) reached −34 kPa and required a drying period of 7 days. In the most severe drying treatment (AWD25), the SWP (0–15 cm) reached −71 kPa and required a drying period of 11 days. Carrijo et al. (2017) in their analysis of factors affecting yields under AWD classified drying periods as severe (below −20 kPa) and mild (greater than −20 kPa). Based on this, both AWD35 and AWD25 are considered severe drying periods, which usually lead to yield penalties (Carrijo et al., 2017). Despite this, yields and yield components were not affected in our study.

One concern with introducing aerobic periods is the potential for nitrogen losses via nitrification and subsequent denitrification. However, N dynamics were not affected by the different water management treatments as plant total N uptake, including the partitioning between fertilizer- and soil-derived N, were similar among treatments in each year (Table 3). Others (e.g., Reis et al., 2018; LaHue et al.,

Table 4
Root length density and specific root length at different soil depths measured prior to (initial) and after (final) the two AWD drying cycles in 2016 (i.e. all measurements taken during flooded soil conditions). Numbers in parenthesis are standard errors of means. Within each root trait, different letters in a column indicate significant differences ($p < 0.05$) between the treatments.

		Initial			Final		
Soil depth (cm)		0–15	15–25	25–35	0–15	15 –25	25–35
Root length density (cm cm^{-3})	CF	6.9 (1.4) a	1.5 (0.5) a	1.5 (0.6) a	17 (6.7) a	1.5 (0.3) a	0.8 (0.03) b
	AWD-Safe	4.0 (0.8) a	1.4 (0.7) a	1.5 (0.3) a	20.7 (2.5) a	1.5 (0.4) a	1.6 (0.2) a
	AWD35	4.4 (0.2) a	1.1 (0.2) a	1.7 (0.5) a	18.9 (1.2) a	1 (0.1) a	1.4 (0.2) ab
	AWD25	6.2 (2) a	1.4 (1) a	1.2 (0.2) a	20 (0.9) a	1 (0.1) a	0.8 (0.04) b
Specific root length (cm mg^{-1})	CF	15.4 (1.1) a	18.1 (4.7) a	14.6 (0.8) a	17.7 (5.5) a	11.7 (0.9) a	12.8 (2.3) a
	AWD-Safe	16.1 (1.5) a	13.4 (2.0) a	21.7 (6.3) a	27.3 (3.9) a	13.6 (1.6) a	19.7 (1.9) a
	AWD35	16.9 (2.0) a	27.3 (8.4) a	17.2 (2.3) a	20.3 (2.3) a	16.6 (6.9) a	21.5 (1.2) a
	AWD25	14.8 (1.0) a	21.3 (4.3) a	14.7 (1.0) a	24.3 (3.4) a	12.5 (2.7) a	14.3 (4.7) a

2016) reported similar findings, and LaHue et al. (2016) attributed the lack of difference in total N uptake on the fact that the drying and reflooding events occurred when there was little mineral N in the soil. Indeed, the decision to delay the first drying event until canopy coverage (about 45 days after planting) was to ensure low soil mineral N levels during the dry down period. Dong et al. (2012) found that AWD triggered N losses via nitrification and denitrification compared to a flooded control; however, those losses were quantitatively insignificant and did not affect total plant N uptake or fertilizer use efficiency. Fertilizer N losses were similar between years and ranged from 30 to 49% (Table 3). These losses are roughly comparable to the 50% reported by Bird et al. (2001), also in California rice systems. Further, the small fraction of ^{15}N recovered at 15–30 cm soil depth suggest that fertilizer loss by leaching was negligible. Rice soils in California are typically heavy clays with low percolation, and nitrate leaching has been shown to be negligible (Liang et al., 2014). These results indicate that if AWD as practiced here is implemented, no additional input of N fertilizer is required.

Carbon isotope composition analysis can be used as an indicator of water stress since stressed tissues often show higher ^{13}C composition and thus lower discrimination against the heavier isotope (lower Δ^{13}C values) as a result of a decrease in stomatal conductance (Lambers et al., 2008). Lower discrimination, represented by Δ^{13}C values, have been reported as a result of drought stress in rice (Centritto et al., 2009; Kondo et al., 2004). Carbon isotope discrimination analysis performed on the vegetative plant tissues showed no differences between treatments. Thus, our results indicate that plants in the AWD treatments did not encounter drought stress, supporting the fact that there was no yield reduction, compared to CF.

Unique and important for understanding the results of this study is the quantification of soil moisture below what is typically considered the rice root zone (0–15 cm). The analysis of GWC in the soil profile during the drying periods indicated that differences in soil moisture across treatments became less with soil depth and at the deepest depth examined (25–35 cm) were not significantly different from each other. Assuming that the soil in the CF treatment was saturated at 25–35 cm during the time the AWD treatments were drying (which all happened after the CF treatment had been flooded for at least 48 days), this soil layer was also saturated in the AWD treatments. Thus, even though during the drying periods in AWD35 and AWD25 the soil was dry at the surface (0–15 cm), it was moist at deeper soil layers.

If deeper soil layers played a major role in supplying water during the drying periods in AWD, there should be a sufficient amount of roots in these layers to meet transpiration demands. We found that while 88% of total RLD were in the 0–15 cm depth, in all treatments there were roots down to 35 cm deep. In upland rice, Kondo et al. (2000) found that maximum water extraction rates per unit length of root can be as high as 0.08 mL/day/cm in parts of the roots exposed to moist soil. Assuming that the same rates can be achieved in lowland systems such as the one in this study, the amount of deep roots (25–35 cm) present in the AWD35 and AWD25 treatments could take up more than 9 mm/day when functioning at maximum rates. Thus, despite representing only 6% of the total RLD, the amount of roots present at 25–35 cm soil depth may have been sufficient to maintain water extraction near normal transpiration rates (Linquist et al., 2015b; Montazar et al., 2017).

These results suggest that while AWD-Safe is indeed a safe practice that can be applied in a range of environments without causing yield reductions (Lampayan et al., 2015), more severe AWD drying periods are possible in certain circumstances. Understanding the hydrology and rooting patterns at deeper soil depths is critical to know whether rice can tolerate drier surface level conditions. Our results suggest that yields were maintained in AWD due to the availability of water at deeper soil layers and the presence of roots in this layer. This may have been caused by a shallow groundwater table and/or by groundwater capillary rise as it has been found to be a significant source of water in aerobic rice systems (Bouman et al., 2007a). This area of the Sacramento Valley has a shallow water table that is less than 1 m below the soil surface (CA DWR, 2017).

4.2. Grain arsenic concentration

The accumulation of As in rice grain is enhanced in CF systems because anaerobic soil conditions increase the bioavailability of As in the soil by: [1] favoring the reduction of As^{5+} to As^{3+}, which is more mobile in the soil, and [2] favoring the reduction of Fe^{3+} to Fe^{2+}, dissolving iron plaques containing As and allowing As to enter the soil solution (Meharg and Zhao, 2012). Further, the reduction of Fe^{3+} to Fe^{2+} also favors the movement of Fe^{2+} from the bulk soil to aerated portions of the root surface causing the formation of new iron plaques that can accumulate As (Yamaguchi et al., 2014). Thus, the potential of AWD to reduce grain As concentration is due to improved soil aeration caused by the introduction of drying periods (Bakhat et al., 2017). The AWD-Safe treatment did not decrease grain As concentration compared to CF likely because the soil moisture at 0–15 cm depth was maintained near saturation throughout the drying periods (Fig. 2A), suggesting that the soil was not aerated enough to decrease soil As bioavailability. Other studies (Arao et al., 2009; Linquist et al., 2015b; Das et al., 2016; LaHue et al., 2016) that reported reductions in grain As concentrations with AWD had AWD treatments that were more severe than the AWD-Safe treatment used here. However, Norton et al. (2017) recently reported a 14–26% decrease in grain As concentrations under AWD-Safe practices. Importantly, depending on the soil and field characteristics, AWD-Safe may cause the SWP to drop to lower levels (down to ≈ -15 kPa) than those seen here (Lampayan et al., 2015), but SWP was not reported by Norton et al. (2017). A more direct determinant of soil As availability is soil redox potential (Eh) (Masscheleyn et al., 1991). For example, Honma et al. (2016) reported that total dissolved As concentration in soil was very low (7.7 $\mu g\ L^{-1}$) when soil Eh was above -100 mV. Establishing relationships between soil Eh and soil moisture may help determine how AWD can be most effective in decreasing As uptake.

While AWD-Safe did not impact grain As concentration in our study, both AWD35 and AWD25 decreased grain As concentration by a similar magnitude of 56%–68% (Fig. 4). In a soil containing four times more As, two AWD treatments that were more severe than the AWD35 and AWD25 treatments decreased grain As concentration by a similar magnitude of 46%–66% (Linquist et al., 2015b). A similar decrease in grain As concentration was observed with an AWD reflooded at 40% of saturated VWC in a soil containing even higher As concentration (Das et al., 2016). These findings suggest that, independent of soil As content, drying the soil further than what was achieved in the AWD35 treatment does not necessarily promote further decrease in grain As concentration.

Although only total As concentrations were determined in this study, it is important to consider As species composition in rice grain since the inorganic forms are more toxic than the methylated (organic) forms (Mandal and Suzuki, 2002). Because demethylation reactions are favored by aerobic conditions, inorganic species usually compose a greater fraction of total As concentration in aerated soils compared to flooded soils, and this pattern is usually reflected in the grain (Meharg and Zhao, 2012). Consequently, reductions in total As observed here with AWD35 and AWD25 may translate into less pronounced reductions in inorganic As, as it has been observed by others (Das et al., 2016).

5. Conclusions

Despite other studies showing that severe AWD, such as the ones imposed here (AWD35 and AWD25), decrease yield compared to continuously flooded systems, yields were not reduced and plants did not encounter water stress. This was likely because water was available deeper in the soil profile (25–35 cm) and the roots in this layer provided sufficient water uptake to maintain plant transpiration. In addition to maintaining yields, the AWD35 and AWD25 treatments decreased grain arsenic concentration by 56–68%. In the AWD-Safe treatment, the least severe of the AWD treatments imposed here, yields were also maintained compared to CF, corroborating with what other studies have observed; however, grain As concentration did not decrease because the soil did not become sufficiently aerobic. These results show that if AWD is to serve as a mitigation practice for grain As accumulation, soils may need to dry further than what was achieved with AWD-Safe. Importantly, these outcomes are not necessarily universal and soil water thresholds observed here may be different depending on the soil type and texture. Understanding the surface and subsurface hydrology and root distribution will help determine the appropriate level of drying severity for AWD practices.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.fcr.2018.02.026.

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