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Irrigation and phosphorous fertilization management to minimize rice grain arsenic content

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HIGHLIGHTS

- Two soil drying events during reproductive stage reduced inorganic arsenic in grain.
- Yield was not affected by drying events or by the level of phosphorous fertilization.
- Alternative irrigation management used similar amount of water than the control.
- Irrigation thresholds established were enough to affect soil pH and Eh dynamics.

GRAPHICAL ABSTRACT

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ABSTRACT

This research sought to minimize inorganic arsenic levels in polished rice grain by using different irrigation and phosphorous fertilization practices while also maintaining crop yield and water productivity. Two experiments were conducted during seasons 2018–2019 and 2019–2020 using a split-plot design with three blocks, five irrigation treatments (main-plots) and two phosphorous levels (sub-plots). Irrigation treatments consisted of a traditional continuous flood (CF) control and four alternatives irrigation techniques with one or two drying events during the irrigation cycle. The phosphorous fertilization levels investigated were an unfertilized control (0 kg P_2O_5 ha^{-1}) and the recommended fertilization level of 50 kg P_2O_5 ha^{-1}. Soil pH and redox potentials were measured in each treatment. Strategically-timed, low severity drying events were effective at achieving aerobic soil conditions, resulting in Eh values over 50 mV. The alternative irrigation treatment with two drying events, implemented at panicle initiation and full flowering, was the most effective in reducing inorganic arsenic in grain without affecting grain yield or the amount of irrigation water applied. This irrigation technique could be considered as an alternative management to the traditional continuous flooded to reach minimal inorganic arsenic accumulation in grain in order to attend special quality standards or specific market requirements. Accumulated inorganic arsenic in grain was below international maximum levels in all analyzed samples, with an average value of 0.084 mg kg^{-1}.

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1. Introduction

Rice is the most important source of carbohydrates for almost half of the world population.

Most of world rice production (75%) over 93 million ha are under continuous flooding irrigation (Rao et al., 2017). In Uruguay, rice is the main irrigated crop, reaching almost 80% of total irrigation area in the country, with 140- to 195-thousand ha annually planted (DIEA MGAP, 2020). Rice seeding starts mainly in October, in dry soil conditions and most crop management operations are done before irrigation is initiated. The most sown varieties are *Indica* type, representing 70% of total rice production area. Rice is irrigated using a shallow, continuous flood within a contour levee-levee gate (i.e., cascade) system. Irrigation normally begins about 15–25 days after crop emergence when plants have 3–4 leaves and begin tillering (Counce et al., 2000). A 5–10 cm water layer is maintained until 10–20 days before harvest (Carracelas et al., 2019a). Crop yields average 8.6-ton ha^{-1}. National paddy rice production is over 1.2 Mt, and more than 95% is annually exported worldwide (DIEA MGAP, 2020). Total water consumption under continuous flooding irrigation ranges from 11.000 to 15.000 m^3 ha^{-1} being 50% of total water consumption apported by rainfall Irrigation period can last in average for 90 days (80–100 days) (Böcking et al., 2008; Ricetto et al., 2017; Carracelas et al., 2019a).

Continuous flooding presents some advantages to rice crop system as a better weed control, higher nutrient availability, reduced disease incidence and protection against low temperatures during microspore formation (Humpreys et al., 2006), which are important to ensure high yields. Considering that only 6000–7000 m^3 ha^{-1} are required by rice evapotranspiration during crop cycle (Blanco et al., 1984; Carracelas et al., 2019a), the interruption of continuous flooding irrigation in short periods at strategic crop stages could lead to a reduction in irrigation water inputs or even to a higher rainfall capture, improving irrigation water-use efficiency. (Massey et al., 2014; Avila et al., 2015).

Arsenic is a harmful element for humans and is associated with diverse health problems as cancer, hypertension, diabetes, premature birth (NRC, 2001; WHO, 2004). Drinking water and rice consumption are two of the major dietary sources of arsenic for human (Meacher et al., 2002; Li et al., 2011; Fu et al., 2011; Meharg and Zhao, 2012; Zhao et al., 2020).

Arsenic in rice grain can be found in inorganic (iAs) and organic (oAs) forms being the first group more toxic for human health. Main iAs species in rice grain are arsenite (As^{III}), and arsenate (As^{V}), while most relevant oAs compounds are monomethylarsonate (MMA) and dimethylarssinate (DMA). Inorganic As in rice in Uruguay have being reported with levels below international regulation. However, there is a permanent interest from the rice industry to develop techniques to satisfy special quality standards or specific market requirements like the baby food sector. In South America arsenic speciation in rice grain can vary greatly depending on the rice producing region (Roel et al., 2021).

Rice is recognized for having a special ability to accumulate As in the grains due to its inherently ability to take up and translocate As into grain in relation to other crops (Islam et al., 2016). Additionally, anaerobic conditions under traditional flood management result in higher As bioavailability in rice fields. (Williams et al., 2007; Su et al., 2010; Meharg et al., 2012). Zhao and Wang (2020) concluded that the concentration of As and cadmium (Cd) in rice grain can vary by three orders of magnitude, depending on bioavailability of these two elements in soil, rice genotype and crop growing conditions. As and Cd bioavailability are both affected by redox potential (Eh, mV) and pH. Lower and even negative values of redox potential that occur under flooding and anaerobic conditions, can determine an increase in As bioavailability while Cd bioavailability will decrease. The suspension of flooding irrigation during short periods can induce soil aerobic conditions by increasing redox potential with the objective of reducing As availability for rice plants. Carracelas et al. (2019b), determined that negative Eh (mV) values could be reached after 50 days of soil continuous flooding for two experimental sites in Uruguay. Arsenic absorption by plants depends on the As speciation: the chemical form As^{V} absorption occurs mainly through phosphate transporters due to its similar chemical characteristics; while As^{III} absorption path is through aquaporins responsible of silicic acid uptake. Phosphates plays an important role in As dynamics in soils competing with As for adsorption sites or Fe-plaque via ligand exchange mechanisms, increasing its bioavailability for plants (Peryea and Kammereck., 1997; Bolan et al., 2013; Wu et al., 2021). On the other hand, when As reaches a critical concentration in soils, As absorption as As^{V} might be reduced by competing for phosphates transporters. Abedin et al. (2002) found that increasing phosphate concentrations in the range from 0.01 to 0.5 mM in the solution of hydroponically grown rice with 0.05 mM of arsenate could reduce As uptake. The decrease in arsenate absorption was higher at higher phosphate concentration. Traditional fertilization of phosphorus in Uruguay consists in the application of 50 units of P at planting. There is a lack of information regarding if this management can affect As grain levels.

The irrigation management technique known as alternate wetting and drying (AWD) applies single or multiple field drying periods, even below saturation, at different crop cycle stages, inducing aerobic conditions to the soil. The increase in oxygen concentration in the rizosphere causes an increase in redox potential, reducing arsenic mobilization (Meharg and Zhao., 2012; Seyfferth et al., 2018). Many benefits related to food safety production and reduction in environmental impacts have been attributed to AWD irrigation techniques, as reduction in As accumulation in rice grain, reduced irrigation water inputs and lower greenhouse gas emissions. However, high variability in results in rice grain yield impact by using AWD has been reported, mainly related with the combination of timing, duration, and severity of soil dryings events when applying this technique (Linquist et al., 2015; Tarlera et al., 2016; Mitra et al., 2017; Yang et al., 2017a,b; Carrijo et al., 2017; Martínez-Eixarch et al., 2021).

Taking in consideration reported variability on rice productivity caused by AWD and the issue of the potential difficulties to implement at large scale rice systems, as in Uruguay, we decided to explore strategic low severity soil drainage at different stages of the crop. Based on existing information, alternative irrigation techniques (AIT) to continuous flooded treatment have been designed to explore the application and combination of short and low severity soil drying periods at specific stages along the whole crop cycle aiming to avoid grain yield penalty (Carrijo et al., 2019).

The primary objective of this paper was to study the relationship between irrigation management and phosphorous fertilization on iAs accumulation in polished rice grain of a long cycle *Indica* variety (INIA Merin). The main hypothesis tested is that drying the field

Field at certain periods and reducing the application of phosphorous fertilizer would reduce inorganic arsenic (iAs) levels in polished rice grain without affecting grain yield compared to conventional practices.

Specific aims of this research were: 1. Determine if alternative irrigation techniques would be effective at modifying chemical properties of soils to reduce iAs bioavailability and accumulation in rice grain, 2. Investigate if by not applying the traditional phosphorous fertilization management of 50 U of P at planting could affect iAs accumulation in polished rice grain.

2. Materials and methods

2.1. Site description

Experiments were conducted in Paso de la Laguna (PdL) at the National Institute for Agricultural Research (INIA) experiment station located in Treinta y Tres, the eastern rice producing region of Uruguay (33°16′11.39″S, 54° 9′58.98″O). ***(Fig. 1)***.

Experiments were carried out during the 2018–2019 and 2019–2020 growing seasons on a soil typical of the main rice producing region of

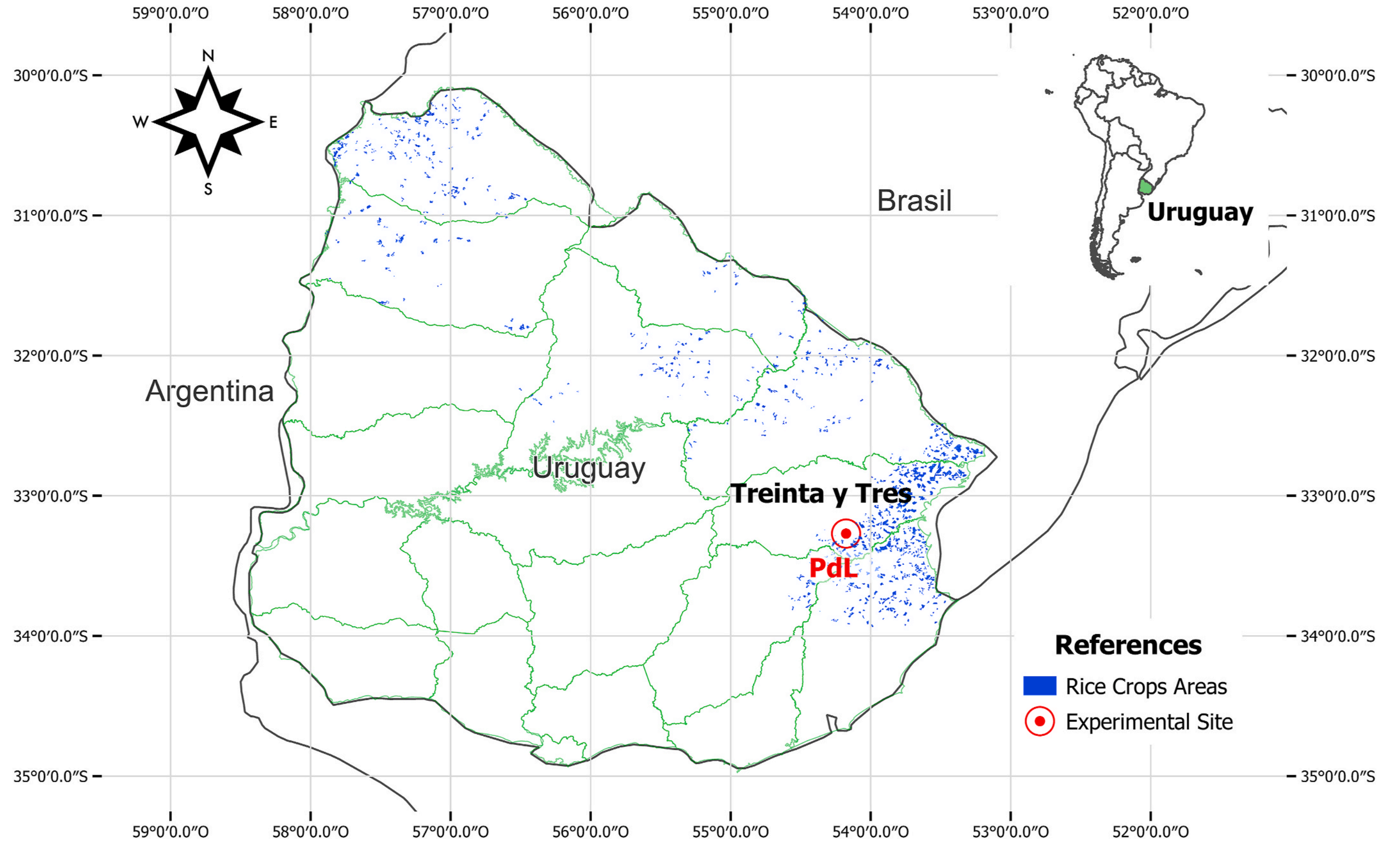

Fig. 1. Rice cultivated area in Uruguay and location of the rice field experimental site of. Paso de la Laguna (PdL) of the National Institute for Agricultural Research (INIA) on East Region of Uruguay.

Uruguay. The soil, Natraquoll (USDA, 1999), is composed of 13.2% sand, 61.0% silt and 26% clay, CEC of 13.5 mg 100 gr $^{-1}$ and pH of 5.8 and 5.5 in seasons 1 and 2, respectively. The presence of a subsurface soil horizon with high content of clay limits rooting depth to 20–30 cm. Organic matter content was 2.24% for both seasons. Phosphorous levels, determined by citric acid method, were 5 ppm. Potassium content was 0.26 and 0.27 meq 100 g^{-1} for the same seasons, respectively. Irrigation water was obtained from Olimar River, a tributary river of Merin Lagoon.

2.2. Field management

The experiments were planted with a long cycle *Indica* cultivar INIA Merin using a Semeato 249 (https://www.semeato.com.br/) direct drilling seeder with 17 cm of row spacing. Sowing dates were 19th of October in 2018–2019 season and 11th of October for the 2019–2020 which are considered as optimal sowing dates for Uruguayan weather conditions (Tseng et al., 2021). Plant sowing density was adjusted according to the germination percentage and the weight of seeds in order to get 500 viable seeds m^{-2}. Triple superphosphate (46% P_2O_5) was the phosphorous source applied after sowing in each subplot according to the treatments and experimental design. In the same way, KCl (60% K_2O) was the potassium fertilizer applied immediately after sowing. Potassium and nitrogen fertilization were defined according to critical levels defined by previous research developed at INIA (Castillo, 2015) and resumed in Fertilizarr software (INIA's technical recommendation software) (http://www.inia.org.uy). Nitrogen was applied as Urea (46% N) twice during the crop cycle: at tillering and panicle initiation stages. Main management practices are resumed in Table 1. Land preparation, weed control and first nitrogen application were all done on dry soil before flooding. The application of nitrogen fertilizer at panicle initiation was done for all treatments on flooded soils, ensuring same

Table 1
General management practices by season and registered precipitations (pp, mm) during soil drying periods of the alternative irrigation techniques (AIT).

	Season			
	2018–2019		2019–2020	
Sowing	Oct 19th		Oct 11th	
Phosphorous fertilization	Oct 19th		Nov 5th	
Potassium fertilization (rate)	–		Oct 11th	(113 kg ha^{-1} K_2O)
Emergence	Nov 6th		Nov 5th	
1st Nitrogen application (rate)	Nov 22nd	(32 kg ha^{-1} N_2)	Nov 25th	(28 kg ha^{-1} N_2)
Initial flood	Nov 27th		Nov 25th	
Vegetative drying (pp)	Dec 13th-26th	(63,6 mm)	Dec 10th-18th	(58,8 mm)
Panicle initiation Drying (pp)	Jan 2nd-8th	(89,6 mm)	Dec 26th-31st	(5,6 mm)
2nd Nitrogen application (rate)	Jan 7th	(43 kg ha^{-1} N_2)	Dec 31st	(35 kg ha^{-1} N_2)
50% Flowering	Feb 8th		Feb 9th	
Full Flowering Drying (pp)	Feb 13th-19th	(44,6 mm)	Feb 14th-20th	(6,1 mm)
Irrigation ending	Mar 6th		Mar 9th	
Harvest	Mar 18th		Mar 31st	

conditions for all irrigation treatments. In plots being dried during this stage, fertilization was done after reflooding to a avoid N losses. Irrigation was terminated two weeks before harvest in all plots.

2.3. Experiment design and treatments description

The experimental design consisted of a split plot design with three blocks. In each block, five irrigation treatments were randomized as the main-plot factor (46 m^{-2}). Plots were separated by levees and drainage ditches. The main plots were divided into two subplots where phosphorous fertilization treatments were assigned randomly (no P application or 50 units of P). Irrigation was started 20–30 days after crop emergence simultaneously in all the treatment (Fig. 2). Continuous flooding (CF, control treatment) and four alternative irrigation techniques (AIT) were tested.

In CF treatment, a 10 cm water layer was kept above the soil surface during the entire irrigation period. In AIT treatments, a 10 cm water layer was kept above soil surface, but plots were drained once or twice at specific crop stages during the season. Vegetative drying treatment (VD) was done 15 days after irrigation started. Panicle Initiation drying treatment (PID) plots were dried when the crop was at panicle initiation stage. Vegetative and Panicle Initiation drying (VPID) treatment plots were dried twice, 15 days after irrigation started and at panicle initiation. Finally, panicle initiation and flowering drying treatments (PIFD) plots were dried twice during crop season, at panicle initiation and full flowering (100% flowering) stages (Fig. 2). Plots were reflooded when a water depletion of 50% of soil available water was reached in the first 20 cm of soil. Soil hydric parameters as: saturation, field capacity, permanent wilting point and available water content were determined by Richard's method (Richards, L.A,1948). The targeted volumetric water content (VWC) value for reflooding was 0.376 m^3 m^{-3}. According to the targeted water threshold, 18 mm of depletion in the first 20 cm of rooting depth was allowed.

2.4. Chemical and crop measured parameters

2.4.1. Total and bioavailable arsenic in soils

Bioavailable arsenic (bioAs) was analyzed for both seasons while total arsenic (tAs) was analyzed only in season 2018–2019. For both analyses, composite soil samples from 0 to 20 cm were taken from the fields 10–15 days before sowing. Bioavailable and total soil arsenic were determined using microwave digestion and inductively coupled plasma (ICP) optical emission spectroscopy, as described Carracelas et al. (2019b).

2.4.2. Redox potential and pH in soil

Redox potential (Eh, mV) and pH were measured weekly using a portable Horiba device equipped with a platinum electrode (LAQUA Act model PH120). Five replicates were taken for pH and redox potential, between the second and third rice row, at 10 cm depth irrigation treatment. In those plots there were also installed frequency-domain (FDR) sensors to measure soil moisture content. Additional Eh and pH measurements were taken 24-h after each reflooding of the plots.

2.4.3. Soil moisture measurements

Soil moisture was monitored using 10 HS (Decagon Devices, Inc, Pullman, WA) FDR sensors connected to EM 50 data loggers (Decagon Devices, Inc, Pullman, WA) which were configured to record soil moisture on an hourly basis. The sensors were installed horizontally at 12.5 cm depth in one plot of each treatment, except for PIFD plot treatment where two sensors were installed at 6 and 18.5 cm to monitor soil moisture when rice plants reached their maximum radical depth. Additionally, gravimetric water content (GWC) was measured at 0–20 cm depth during drying periods. GWC samples were taken 24-h after each drainage period started, and samples were obtained every 2–3 days to follow soil moisture evolution, being the last sampling done immediately before each reflooding event to determine lowest soil moisture. Soil GWC was determined taking three samples on each plot from 0 to 20 cm. Each sample was partitioned and pooled into two soil depths, 0–10 cm and 10–20 cm. All samples were dried to 105 °C until constant weight. Soil GWC was then calculated using Equation 1, where W = sample wet weight, D = sample dry weight.

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

Bulk density was determined using a 4.5 cm diameter soil core from 0 to 10 cm, 10–20 cm and 20–30 cm of soil depth. Undisturbed bulk density samples were oven-dried at 105 °C until constant weight. Finally, volumetric water content (VWC) was calculated multiplying GWC and bulk density. Soil available water storage capacity was determined as the difference between VWC at field capacity and VWC at permanent wilt point (Richards, 1948).

2.4.4. Inorganic arsenic in polished rice grain

Determination of iAs in rice grain was done in Technological

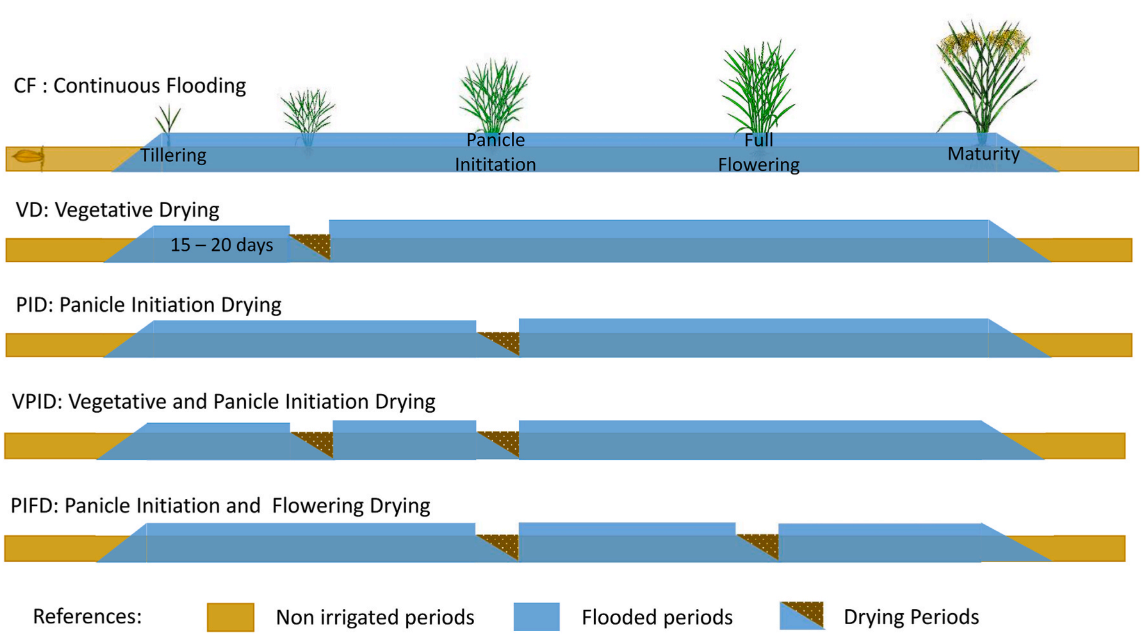

Fig. 2. Irrigation treatments evaluated during two crop seasons (2018–2019 and 2019–2020). CF: Continuous flooded, AIT treatments were VD: Vegetative drying, PID: Panicle initiation drying, VPID: Vegetative and panicle initiation drying, PIFD: Panicle initiation and 100% flowering drying.

Laboratory of Uruguay (LATU) following the same procedure described by Roel et al. (2021).

2.4.5. Cadmium in polished rice grain

Twelve rice grain samples (two from each of the three blocks) of most contrasting irrigation treatments CF and PIFD (total n = 12) were selected in order to analyze cadmium concentration in both sites in the first season. Polished rice grain samples were ground with a blade mill to pass a 1 mm sieve. Next, 0.3 g milled rice was digested with 3.0 mL of Nitric Acid (Merck, 65% for analysis) and 2.0 mL of Hydrogen Peroxide (30% w/v) in a microwave (Milestone, Ethos One, Italy) and the digests were diluted to 50 mL with Nitric acid 0,5% in deionized water. Inductively Coupled Plasma-Mass Spectrometry was used to determine Cd (Nex Ion 350 D, Perkin Elmer, USA). Calibration curves were prepared with cadmium (1000 mg L^{-1}) stock standards from Inorganic Venture (USA). Every forth sample, one blank, two fortified samples, and one certified reference standard (1568 b Rice Flour, National Institute of Standards and Technology, USA) were included as quality control samples. The certified reference material (1568 b) was used to assess the accuracy of Cd concentration for rice flour.

2.4.6. Irrigation water inputs

Irrigation water inputs (WIi) were measured with helicoidal flowmeters (ARAD, WMR50) at the entrance of each plot to allow independent management according to each irrigation treatment. Water was pumped from nearby irrigation channels to ensure full-pipe water flow. Water inputs were then adjusted to m^3 ha^{-1}.

2.4.7. Grain yield

Grain yield was obtained by manual harvest of 4.08 square meters (8 rows x 3 m) from the center of each plot when a grain moisture of 21% was reached. Samples were mechanically threshed, and grain yields were corrected to 13% moisture. Harvested samples were meticulously identified and carried to INIA's grain laboratory where they were dried at 60 °C until 13% moisture was reached. Grain subsamples identification was codified and As grain content analyses were performed by an independent laboratory (LATU).

2.5. Statistical analysis

All statistical analysis was performed using R software (R Core Team, 2019) in combination with nlme, emmeans, ggplot2 packages. For the response variables yield and iAs, a linear mixed effect model was used. Analyses of variance was performed followed by means separation using Tukey's test. Fixed effects considered were: season, irrigation and phosphorous fertilization treatments and the interaction between season and irrigation treatments. Random effects were: block and main plot. For the response variables WIi, the same procedure was performed

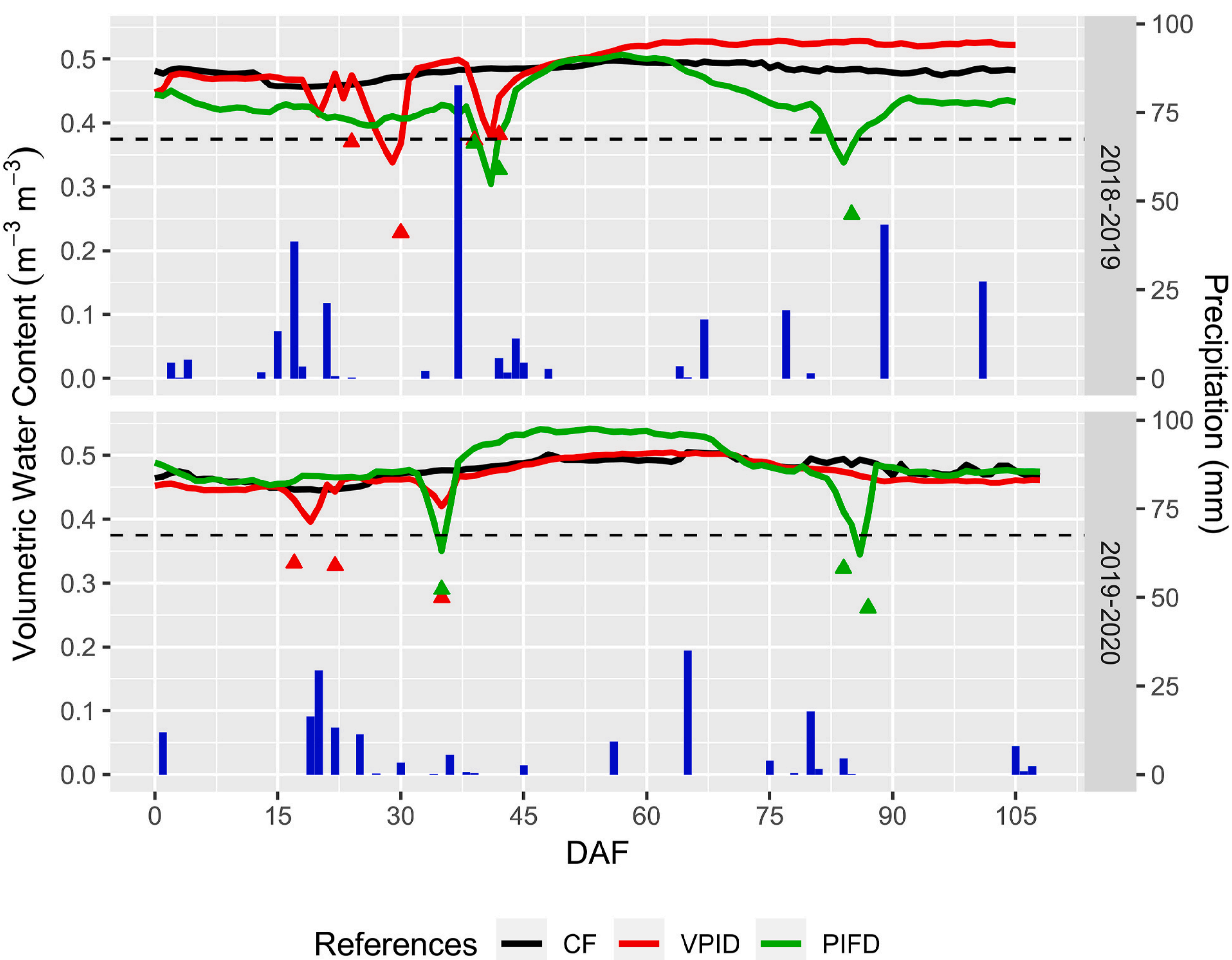

Fig. 3. Soil volumetric water content registered along days after flooding (DAF) in PdL experiments for most contrasting irrigation treatments across two crop seasons: 2018–2019 and 2019–2020. Lines represents frequency-domain sensor (FDR) records and triangles represents gravimetric water content for Continuous flooded (CF, black), Vegetative and panicle initiation drying (VPID, green) and Panicle initiation and full flowering drying (PIFD, red) irrigation treatments. Blue bars represent precipitation events. Black dashed line represents irrigation threshold in order to reflood treatments. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

defining season, irrigation and the interaction between them as fixed effects while block was defined as random effects.

3. Results

3.1. Soil moisture

Volumetric water contents measured by the FDR sensors and the VWC values calculated from gravimetric samples taken at the different drying periods and precipitations during irrigation period are represented in ***Fig. 3***.

Precipitations records during irrigation period were 282 and 213 mm for season 1 and 2 respectively, and were lower than historical average of 392 mm registered during the last 50 years. The duration of each drying period was around 5–7 days when this period was not interrupted by precipitation. Average VWC records from FDR sensors in CF treatment were 0.481 and 0.478 m^3 m^{-3} for first and second season respectively. VWC in CF treatment never dropped from saturation (***Fig. 3***). Drying periods applied along crop cycle are represented. Vegetative drying period started around 20 DAF in both seasons. Panicle initiation drying period was done 35–40 DAF. Finally, full flowering drying period was done at 80 DAF for both seasons. The targeted irrigation threshold was reached for each of the three drying periods in both seasons.

3.2. Redox potential and pH in soil

Evolution of redox potential (Eh, mV) for both seasons is represented in Fig. 4. Positive Initial redox potential values were measured in a range between 100 and 250 mV for all treatments across seasons. After initial flooding, Eh shows a decreasing trend in both seasons. Control CF treatment reported negative values 15 DAF with a similar behavior in both seasons. Negative values were recorded in this treatment for the rest of the irrigation period. When irrigation was initiated, VPID treatment follows the same decreasing trend until first drying was imposed. Positive Eh values over 150 mV were reached during this drying event. A decreasing Eh trend was observed after reflooding this treatment, followed by an increment up to 150–250 mV at 35–45 DAF when the second drying period was implemented. Similar. positive redox potential values were observed at panicle initiation stage in PIFD treatment with

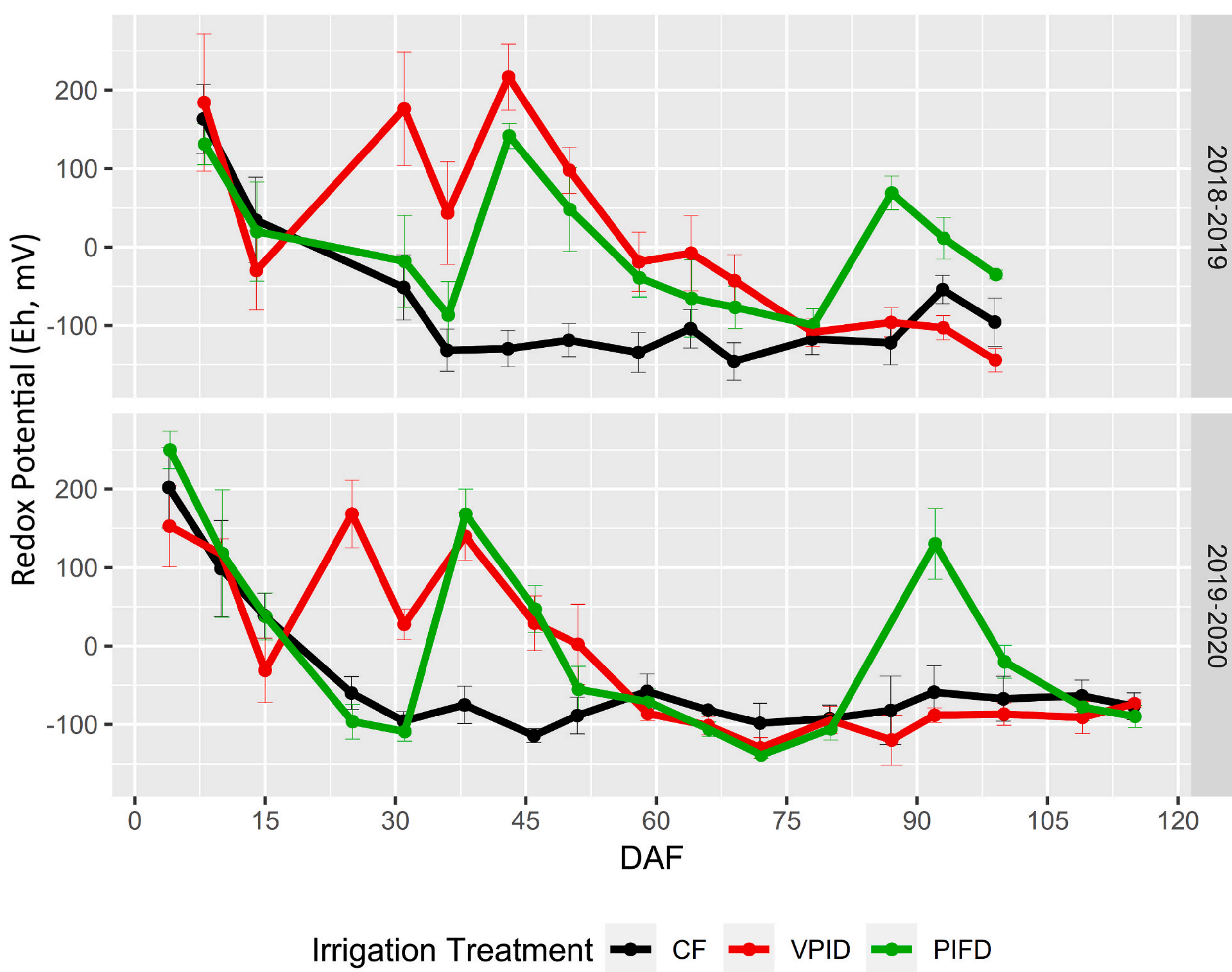

Fig. 4. Redox Potential (Eh, mV) trend along days after flooding (DAF) for Continuous. flooding (CF, black line), Vegetative and panicle initiation drying (VPID, red line) and Panicle initiation and full flowering drying (PIFD, green line) treatments in Paso de la Laguna (PdL) experimental site, across two seasons: 2018–2019 and 2019–2020. Bars represent standard errors of means. Evolution of pH for both seasons is represented in Fig. 5. Initial pH values were between 5 and 6 in both seasons. After initial flood was stablished, pH values were increased in CF tending to neutrality. Around 30 DAF, pH values in CF stayed between 6 and 7 until harvest. In VPID treatment, the tendency after initial flood was the same as in CF treatment, except for measurements taken immediately after vegetative and panicle initiation drying events when pH tends to decrease and pH values were lower than obtained in CF. In PIFD the initial tendency was to increase pH values until panicle initiation and full flowering drying events were applied. pH measurements taken immediately after these two drying events, (40 and 80 DAF) showed lower pH values than CF, being more evident at panicle initiation drainage. . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

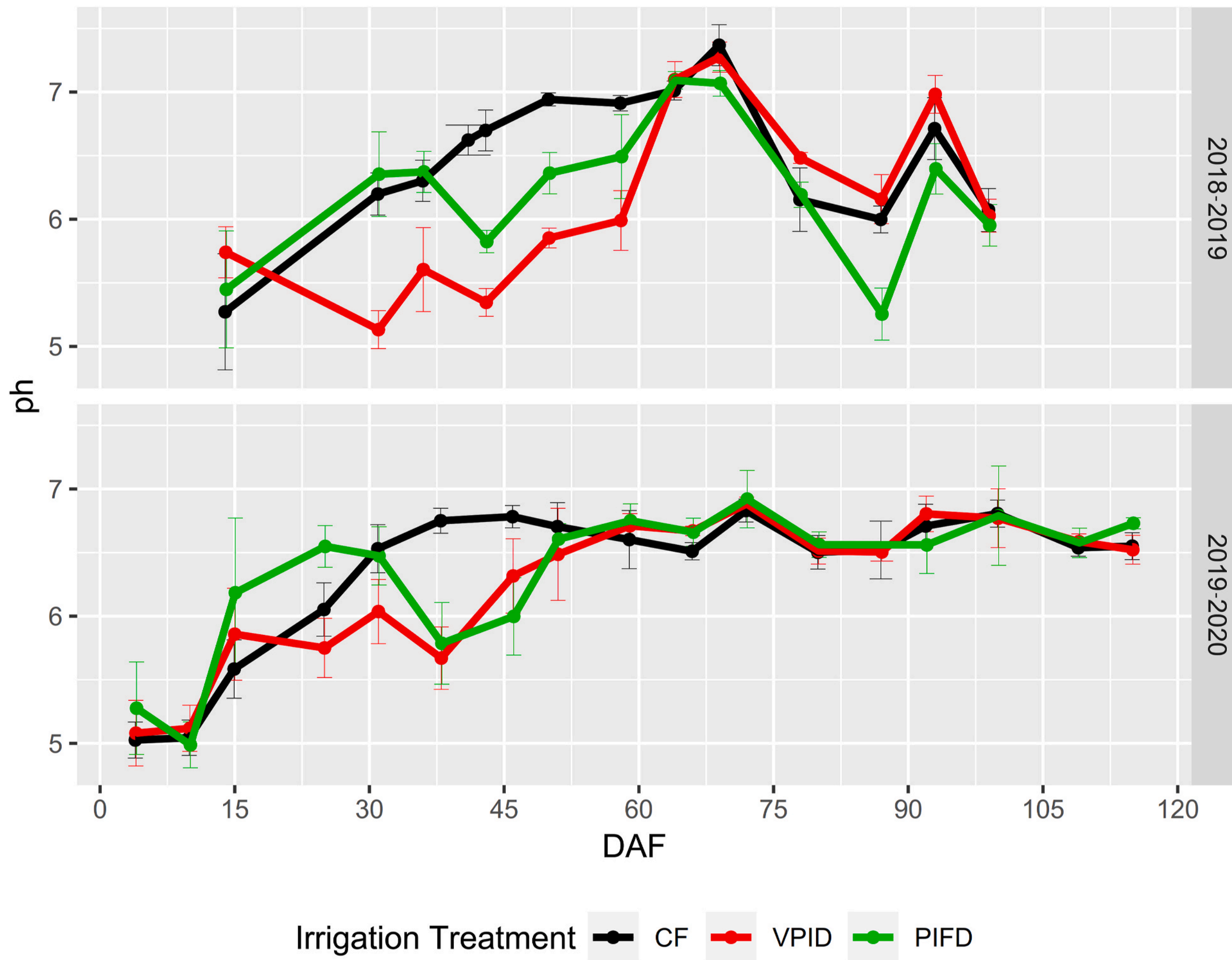

Fig. 5. pH evolution along days after flooding (DAF) for Continuous flooding (CF, black line), Vegetative and panicle initiation drying (VPID, red line) and Panicle initiation and full flowering drying (PIFD, green line) treatments in Paso de la Laguna (PdL) experimental site, across two seasons: 2018–2019 and 2019–2020. Bars represent standard errors of means. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

later decreasing values as in VPID, followed by a peak observed at 90 DAF immediately after full flowering drying period was applied.

3.3. Total and bioavailable arsenic in soil

Total arsenic (tAs) level in soil at sowing in the first season was 3.506 mg kg^{-1}. Bioavailable As (bioAs) levels in soil at sowing were 0.175 and 0.26 mg kg^{-1} of dry soil in seasons 2018–2019 and 2019–2020, respectively.

3.4. Rice grain yield

Rice grain yield mean value (13% moisture) for both seasons was 10,921 kg ha^{-1} with a coefficient of variation of 7.5% (Table 2). Significant differences between seasons were detected, with a mean grain yield of 10,490 and 11,352 kg ha^{-1} in seasons 1 and 2, respectively. No other statistically significant effects were detected for grain yields. Statistical differences were detected for the interaction between irrigation and phosphorous treatments but mean yield values were not different using Tukey's test.

Means followed by different letters are significantly different with a probability less than 5% ($P < 0.05$). $P < 0.001$ $P <$ NS: non-significant differences. CV: coefficient of variation.

Table 2
Rice grain yield (kg ha^{-1}, 13% moisture) and Inorganic arsenic concentrations (mg kg^{-1}) in 2018-2019 and 2019–2020 seasons from Paso de la Laguna (PdL) experimental site in Uruguay, by five irrigation treatments in INIA Merin Variety.

Classification criteria Season	Rice Yield (kg ha^{-1})	iAs (mg kg $^{-1}$)
2018–2019	10,490 [b]	0.075 [b]
2019–2020	11,352 [a]	0.094 [a]
Average	10,921	0.084
CV%	7.55	18.2
P < 0.05	***	***
Irrigation	10,694	0.086 [a]
Continuous flooding (CF)		
Vegetative drainage (VD)	11,470	0.089 [a]
Panicle initiation drainage (PID)	11,138	0.090 [a]
Vegetative and panicle initiation drainage (VPID)	10,680	0.090 [a]
Panicle initiation and flowering drainage (PIFD)	10,624	0.067 [b]
P < 0.05	NS	***
Phosphorous fertilization		
0UP	10,845	0.085
50UP	10,997	0.084
P < 0.05	NS	NS
Irrigation * Phosphorous fertilization		
P < 0.05	*	NS

3.5. Inorganic arsenic in polished rice grain

The average value for iAs in polished rice grain for both seasons was 0.084 mg kg $^{-1}$ with a coefficient of variance of 18.2% (Table 2). Season and irrigation treatment were significant for iAs while phosphorous fertilization and the interaction between irrigation and phosphorous were not significant. Mean values for iAs levels were 0.075 and 0.094 mg kg^{-1} in seasons 1 and 2, respectively, with former being significantly lower than the latter. The lowest iAs accumulation was associated with the PIFD treatment with a mean value of 0.067 mg kg^{-1} (*Fig. 6*).

3.6. Cadmium in polished rice grain

From the total number of twelve polished rice grain samples analyzed, nine of them presented Cd levels below the method detection level limit of 0.01 mg kg^{-1}. In the three remaining samples, Cd was detected but its concentration was below the quantification level of 0.03 mg kg^{-1}.

3.7. Irrigation water inputs

The mean value of total irrigation water was 10,423 m^3 ha^{-1} for all irrigation treatments and seasons with a coefficient of variation of 14.3% Significant differences were detected between seasons with an average value of 9396 m^3 ha^{-1} for 2018–2019 and 11,449 m^3 ha^{-1} for 2019–2020. No interaction between irrigation treatments and seasons were detected.

4. Discussion

4.1. pH and redox potential

pH and redox potential were modified by alternative irrigation techniques compared to continuous flooding (Figs. 4 and 5). Negative Eh values were reached 15–20 days after initial flooding, in all treatments, similar to what was reported by Tarlera et al. (2016) and Carracelas et al. (2019b) in Uruguay. According to international research, during that time gap, As that was coprecipitated as Fe oxyhydroxides dissolves and its bioavailability increases when reduction of Fe^{III} to Fe^{II} occurs in the Eh range of 0–100 mV (Masscheleyn et al., 1991; Yamaguchi et al., 2011; Zhao et al., 2020). Honma et al. (2016) demonstrated that dissolved As in soil solution is almost linearly related to dissolved Fe in soil solution. Strategic low severity drying events were effective to turn soil into aerobic conditions reaching positive Eh values over 50 mV, and even over 150 mV in most drying periods, what is expected to reduce As mobility and availability in soils (Fig. 4).

At the beginning of irrigation, pH values in CF treatment tend to increase from original acidic values between 5 and 6 to almost reaching the neutrality, being stabilized in a range between 6.5 and 7 around 30 to 45 DAF. In the first season initial increasing tendency is similar, but pH values were more unstable from 45 DAF to irrigation ending. After drying events, pH tends to decrease. Different studies show that As bioavailability is increased by pH values over 6.3 (Masscheleyn et al., 1991; Honma et al., 2016; Zhao and Wang., 2020). This situation generates contrasting soil conditions between irrigation treatments. While in continuous flooding treatment as availability in soil should be increased during the whole irrigation period, in the alternative irrigation techniques treatments, a reduction in As availability should be noticed by the combined effect of pH modifications and redox potential, as illustrated in Figs. 4 and 5.

4.2. Total and bioavailable arsenic in soils

Total As level in soil was measured only in the first season with an average value of 3.51 mg kg^{-1}, similar and slightly lower than the 5 mg kg^{-1} average obtained by Verger at al., 2015 in a 20 soils sampling of the rice producing regions of Uruguay. Carracelas et al. (2019b); reported tAs levels of 3.62 mg kg^{-1} and 2.14 mg kg^{-1} for two sites in the East and

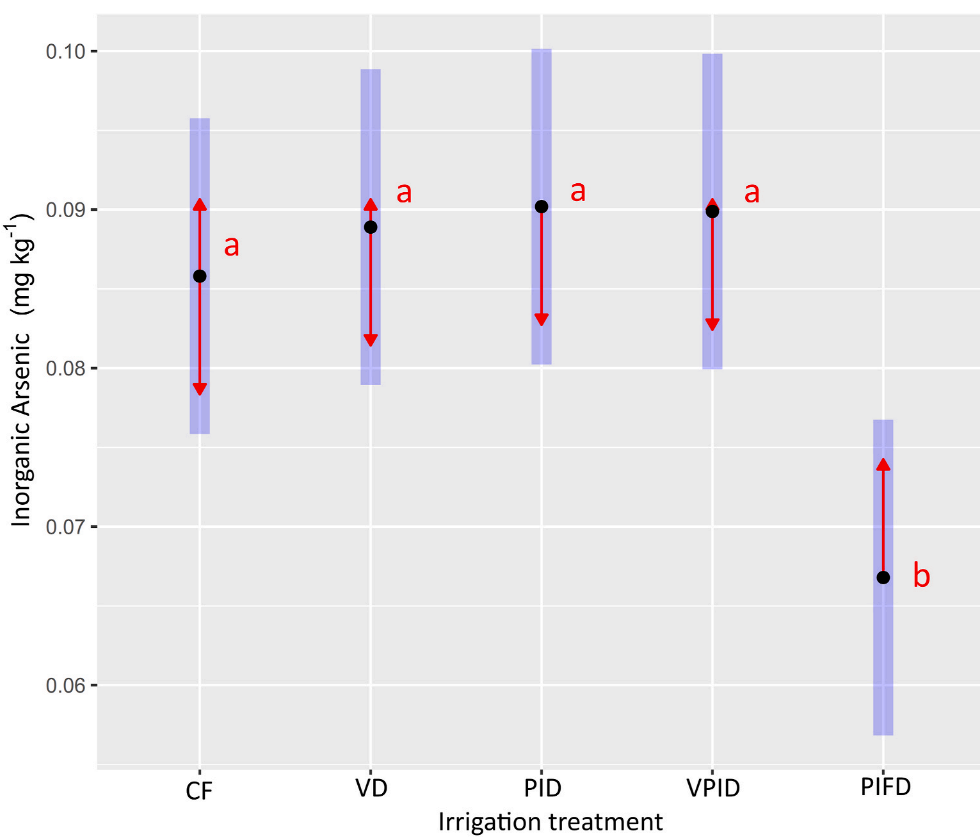

Fig. 6. Inorganic arsenic (iAs) content in polished rice grain (mg kg $^{-1}$) for the different irrigation treatments. CF: Continuous flooded, AIT treatments were VD: Vegetative drying, PID: Panicle initiation drying, VPID: Veg. and PI drying, PIFD: Panicle initiation and full flowering drying in Paso de la Laguna (PdL) experimental site. Black dots represent means, red arrows are indicating confidence intervals by Tukey test for the estimated marginal means and blue bars indicates standard errors. Different letters indicate significant differences with a probability less than 5%. . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

North regions of Uruguay. Quintero et al. (2014); in soils from Entre Rios province, a rice producing region in Argentina located at west from Uruguay river, found tAs levels ranging from 1.6 to 4.1 mg kg^{-1}. The results obtained in actual study are lower than worlds average of 5 mg kg^{-1} (Koljonen et al., 1989) and well below Canadian Environmental Quality Guidelines limit (CCME, 2019) of Arsenic in soil of 12 mg kg^{-1}.

Bioavailable As in soil was 48.6% higher in the second season compared to the first one, with values of 0.14 and 0.21 mg kg^{-1} respectively. The high variability in bioAs might be related to important environmental effect generating such differences within seasons and should be studied deeper. Verger et al. (2015) incubated 10 soils of Uruguayan rice-producing regions under different crop rotations and managements. Maximum As bioavailability was registered between day 5–15 after incubation started for all soil samples, and the tendency was to decrease after reaching that peak. A ten-fold time difference was detected between maximum and minimum bioAs levels during that peak with a range of 0.0175 mg kg^{-1} to 0.1610 mg kg^{-1} of dry soil. After the 5–15 days of incubation peak, bioAs lowered and was stabilized between 0.0120 mg kg^{-1} and 0.0900 mg kg^{-1}. Bioavailable arsenic levels at sowing reported by Carracelas et al. (2019b) in Uruguay were 0.1515 mg kg^{-1} for Paso de la Laguna and 0.0760 mg kg^{-1} for Paso Farias (North region), which are also in consonance with this study.

4.3. Inorganic arsenic in polished rice grain

The mean and range of iAs concentration in polished rice grain of 0.084 mg kg^{-1} (0.050–0.113 mg kg^{-1}) are similar to previous studies developed in Uruguay by Carracelas et al. (2019b) and aligned to what was reported by Roel et al. (2021) for this cultivar. These values are below international Codex Alimentarius (FAO and WHO, 2019) maximum levels for inorganic arsenic in polished grain (0.20 mg kg^{-1}) and 76.7% of samples were below EU (COMMISSION REGULATION, 2015) and USA (FDA, 2020) maximum levels allowed for rice used for preparation of infant food (0.10 mg kg^{-1}). Higher average iAs accumulation was found in second season compared to the first one (0.094 vs 0.075 mg kg^{-1} respectively) that might be explained by higher bioAs determined in soils (Xu et al., 2008; Carracelas et al., 2019b). Alternative irrigation techniques that applied drying periods during vegetative and panicle initiation were not effective to reduce iAs in grain. However, PIFD treatment that combined two drying periods during panicle initiation and full flowering was effective in reducing grain iAs content by 22.1% compared to CF treatment. The combination of severity, timing and number of soil drying periods are relevant aspects when designing irrigation management strategies to reduce As accumulation in grain. According to Carrijo et al. (2019), more than one drying period is necessary to minimize grain As levels when applying low severity soil dryings, which is aligned with the results obtained in this study. Similar irrigation management strategies can reach a reduction in grain arsenic between 16 and 35% (Islam et al., 2017; Norton et al., 2017). As iAs is more toxic than organic forms, and the relation between inorganic and total arsenic not responding to a fixed factor, it seems that future studies should be focused on how irrigation management specifically affects As grain speciation and iAs accumulation. Despite iAs levels that were low in both seasons of the present study, results show that is possible to reduce it even more applying two soil dryings during panicle initiation and full flowering stages as in PIFD treatment. Similar results were reported by Arao et al. (2009); and Zheng et al. (2011). According to these authors iAs transport and accumulation in grain mainly occurs after flowering stage during grain filling. Other combinations of aerobic cycles during irrigation period as alternate wetting and drying irrigation techniques have been tested in previous

Studies in Uruguay as an effective strategy to minimize iAs accumulation in grain (Carracelas et al., 2019b) achieving reduction of 39.6% but affecting grain yield in some cases. Several environmental factors have been reported by international literature as relevant factors in As absorption by plants and might me explaining the 25% higher accumulation in 2019–2020 compared to 2018–2019. Arao et al. (2018) studied the relationship between many climatic factors and iAs accumulation in grain, and discovered that average daily mean and average daily minimum air temperature from 2 to 4 weeks after heading were significantly and positively correlated to iAs in grain in a plot study in Japan. In contrast to that study, average mean temperature in this study for both seasons was 21.6 °C, and average minimum temperature was lower in season 2019–2020 compared to 2018–2019 (13.72 °C vs 15.42 °C respectively). Therefore, specific deeper research should be done to improve the knowledge about environmental and climatic factors over As accumulation in grain. Phosphorous fertilization was not effective as an arsenic accumulation mitigation management alternative.

Some authors affirms that an increase in phosphorous concentrations in soils can increase As concentration in soil solution when P competes by absorption sites in soils or Fe-plaque, increasing As bioavailability until a critical soil P concentration is reached and competition for uptake paths with arsenate occurs, reducing As uptake (Peryea and Kammereck, 1997; Geng et al., 2005; Bogdan et al., 2009; Meharg and Zhao, 2012; Azam et al., 2016; Mitra et al., 2017). However, a high variability in the response of As uptake under different levels of soil P has been reported. Results of this study are similar with what had been reported by Xu et al. (2008) and Wu et al. (2011). They found no relation between soil P and As uptake since the iAs form absorbed by rice plants under flooding conditions is arsenite. Phosphate addition is expected to reduce arsenate absorption through arsenate transporters, not arsenite.

4.4. Cadmium in polished rice grain

Minimizing iAs absorption and accumulation in rice grain applying aerobic cycles during irrigation period could cause a negative effect in Cd accumulation in grain. The increase in Eh and lower pH values could lead to higher Cd availability for plants in soils and higher accumulation in rice grain (Zhao and Wang., 2020). Taking that in account, it was reasonably to analyze Cd concentration in polished rice grain of the most contrasting irrigation treatments. CF treatment as the most anaerobic soil conditions, with lower Eh and more basic pH values in soil solution and PIFD treatment with more aerobic conditions, higher Eh and more acidic pH values. Cd levels in grain were well below Codex Alimentarius maximum levels (0.4 mg kg-1) in all analyzed samples even applying two drying events during crop season. These results are very relevant and encouraging considering that is possible to apply AIT to obtain minimal iAs content in grain with no significantly Cd increase.

4.5. Rice grain yield

Average yield of this study was 10,921 kg ha^{-1}, with 8.2% higher yields in season 2019–2020 compared 2018–2019. These results are consistent with average commercial Uruguayan East rice-producing region yields of 8520 kg ha^{-1} (DIEA MGAP, 2021) and 9350 kg ha^{-1} (DIEA MGAP, 2021a) obtained in seasons 2018–2019 and 2019–2020 respectively. Better climate conditions were registered in season 2019–2020 compared to 2018–2019. According to the information obtained from INIA's climate station located in PdL, sunshine hours accumulated during crop cycle in 2019–2020 were 344 h compared to 221 in 2018–2019. Also, higher tank evaporation registers occurred in season 2019–2020 (120 mm vs 113 mm). Many international studies report contradictory effects on grain yield of alternative irrigation techniques to the traditional continuous flooding system. Linquist et al. (2015), in a two-season study in Arkansas, USA, applied AWD during vegetative stage, with no yield penalty. On the other hand, when AWD was applied during vegetative and reproductive stages, grain yield stability was affected, especially with more severe field dryings. Capurro et al. (2015) in a three years plot study developed in Paso de la Laguna, Uruguay, found that AWD applied during vegetative stage with an irrigation threshold of 50% of available water holding capacity affected grain yield stability, obtaining lower yields than CF treatment in one of the three seasons. A yield loss of 15% was reported by Carracelas et al. (2019a) with similar type of irrigation treatment.

An important result of this study is the feasibility of AIT without crop yield penalization. Alternative irrigation techniques treatments in actual study were designed with specific low severity soil dryings to minimize iAs accumulation in grain avoiding grain yield affection. Carrijo et al. (2018); in California, USA, found that the appliance of two soil dryings at vegetative and panicle initiation stage, reflooding before 50% heading had no effect in grain yield independently of the severity of field dryings. In a later study, Carrijo et al. (2019); determined that the imposition of a single soil drying period within the growing season can mitigate As accumulation in rice grain, but it depends on the severity and timing of the drying period. Drainage during booting and heading were the more effective stages to reduce iAs with no grain yield reduction.

Finally, phosphorous fertilization didn't have a significant effect on grain yield. This situation could be explained by phosphorous levels at soils in both seasons that were above critical levels for this crop according to Hernández et al. (2013) for this region.

4.6. Irrigation water inputs

The only significantly differences in irrigation water inputs detected were associated to the season effect, 9396 m^3 ha^{-1} and 11,449 m^3 ha^{-1} average total irrigation water use in all treatments for season 2018–2019 and 2019–2020, respectively. Lower total water amounts registered in season 2018–2019 were associated with higher amount of precipitation registered in that season. Precipitation during irrigation period 2018–2019 were 32% higher compared to 2019–2020, with 282 mm and 213 mm for each season, respectively. Different alternative irrigation treatments evaluated did not vary significantly in the total amount of irrigation water used. AIT treatments presented similar amount of water use than the continuous flooding treatment (control), indicating that the drainage and reflooding effects did not altered significantly the total amount of irrigation water required (Supplementary Table 1).

5. Conclusions

Combinations of low severity drainages at different rice growth stages were able to alter soil redox potential and pH behavior compared to the traditional continuous flooding management. Strategic low severity drying events were effective to turn soil into aerobic conditions reaching positive Eh values in most drying periods, what is reported to reduce soil As mobility and availability. Levels of P fertilization had no impact on iAs grain content. The hypothesis that by not applying P will potentially reduce soil As availability was not confirmed. This study shows that there is an alternative water management strategy that consisted in applying two strategic low severity drainages at panicle initiation and full flowering stages that allows a significant reduction of inorganic arsenic content level without penalizing yield. Similarly, Cd rice grain levels that can potentially increase under this irrigation treatment were well below Codex Alimentarius maximum levels (0.4 mg kg^{-1}) in all analyzed samples. These results fulfill the interest from the rice industry to develop techniques to satisfy special quality standards or specific market requirements like the baby food sector.

Further validation should be done at farmer scale to evaluate the feasibility of the application of this irrigation management alternative. Additionally, the effect of a single full flowering drying event over iAs accumulation in rice grain, not evaluated in this study, should be addressed in future research.

This study addressed only the inorganic content of Arsenic in rice, while this is the most toxic component, a relevant aspect that should be also taken in consideration are the organic and total component of this element.

Author statement

F. Campos: Methodology, writing, analysis, Investigation, **A. Roel**: Conceptualization, Methodology, Investigation, analysis, writing, **G. Carracelas**: Methodology, analysis, **M. Verger**: Methodology, analysis, **R. Huertas**: Methodology, analysis, **C. Perdomo**: Conceptualization, Methodology, analysis, Investigation

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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