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Evaluating the impacts of water management on arsenic uptake in rice and soil properties

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Evaluating the impacts of water management on arsenic uptake in rice and soil properties

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GENERAL ABSTRACT

Arsenic (As) is a toxic element that exists naturally in the environment in several inorganic and organic forms. Some agricultural activities, such as pesticide application or irrigation with contaminated water have significantly increased As levels in soil in many parts of the world, affecting rice-producing countries. In flooded paddy soils As mobility is high and is readily taken up by rice and accumulated in grain, posing a potentially significant health risk. Water management in rice fields is one of the best approaches in controlling arsenic bioavailability in paddy soils. The water drying events induce redox fluctuations, affecting iron (Fe) and As transformations. Under flooded conditions, rice oxygenates its rhizosphere which precipitates iron oxides on the root surface, forming an iron plaque. The iron minerals present in this plaque have a high affinity for binding to cations and anions, thus having the potential to sequester arsenic and mitigate its uptake into grain.

The implications of water management practices on As uptake in rice and mobility in the soil-root interface of have not been thoroughly characterized. In this study we performed a field-scale experiment during the 2017 and 2018 summer growing seasons, as well as plant growth bioassays with three single-drain irrigation treatments of different soil drying severities (high, medium and low), and a continuously flooded (CF) control, with the objective of reducing As accumulation in rice and determining changes in the rhizosphere. Single drain intermittent irrigation (II) demonstrated potential for on-farm use at field-scale. Yields were maintained and As concentrations decreased by an average of 45%. Cadmium was controlled with a medium severity treatment.

Moreover, to understand the mechanisms involved in arsenic immobilization in the soil-root interface with II, rice root plaque formation and trends in Fe and As accumulation were studied. The introduction of oxic conditions with II favored the formation of ferrihydrite, which possesses a great affinity for binding As and is known to be present consistently in samples of II treatments. Although this mineral

may serve as a sink for As, higher concentrations and accumulation of As was attributed to the CF treatment, elucidating that, under II, the main mechanism for reducing As accumulation in grain is the oxidation and immobilization of As in bulk soil.

Lastly, our analysis of the microbial 16S rRNA gene in rhizosphere samples revealed that the introduction of oxic conditions increases the abundance of aerobic bacteria that contribute to the oxidation and precipitation of Fe in the rhizosphere, and consequently As immobilization. Increasing the severity of II treatments induces a greater shift in the rhizosphere bacterial community of rice.

Understanding the chemical and biological interactions affected by water management treatments in rice systems is a step forward towards defining an effective strategy to minimize As mobility in rice fields that is potentially acceptable by rice growers, which may positively impact food security and human health globally.

CHAPTER 1

Changes in rice arsenic uptake under single-drain intermittent irrigation

Abstract

In many parts of the world arsenic (As) is present in soils and groundwater at elevated concentrations which exceed the generally accepted contaminant threshold values. Growing rice in soils with high levels of As represents a hazard for human health. In flooded paddy soils As mobility is high and is readily taken up by rice and accumulated in grain. Intermittent irrigation (II) is a potential strategy to reduce As concentrations in rice grain that involves drying paddy soils in the middle of the growing season with subsequent reflooding. We performed a field-scale study during the 2017 and 2018 summer growing seasons where three single-drain irrigation treatments with different soil drying severities were compared to a continuously flooded (CF) control. Our results show that II treatment of high severity reduced As concentrations in grain by an average of 45% for both years in comparison to continuously flooded conditions, however, inorganic As was reduced by only 27%; additionally, cadmium (Cd) concentrations in grain increased with II. It was also determined that grain nutrition was not negatively impacted by II treatments. Overall, we demonstrated that single drain II treatments can sustain yields and reduce As accumulation, and a medium severity treatment can balance the accumulation of As and Cd. Our field-scale data is a step forward towards defining an effective strategy to minimize As mobility in rice fields that is potentially acceptable to rice growers.

1. Introduction

Rice is the primary staple food for half of the world's population (Mitra et al., 2017); however, it a potential source of arsenic (As) exposure to humans (Panthri and Gupta, 2018). The main source of As in soil and groundwater is through weathering of As bearing rocks. Additionally, some agricultural activities, such as historical pesticide application or irrigation with groundwater containing high levels of As, have significantly increased As contamination in soils for many parts of the world (Hu et al., 2007).

Arsenic is a toxic metalloid that exists in the environment in both inorganic and organic forms (Mitra et al., 2017). As mobility in agricultural fields and toxicity to humans is largely determined by its oxidation state and chemical form. Inorganic As (*i*As) exists in two oxidation states, +3 and +5, and are the dominant As species in the environment. Arsenate (As^{V}) is the oxidized form and has low mobility in soil, whereas arsenite (As^{III}) is the reduced form predominating under anoxic conditions and has greater mobility and toxicity to humans (Abedin et al., 2002; Bose and Sharma, 2002). The two organic forms of As found in paddy soils are monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA), which form via microbial methylation of *i*As species and are typically considered non-toxic (Abedin et al., 2002; Meharg, 2004).

Rice is typically grown under flooded conditions which have low oxygen levels that lead to reducing conditions. As a result, rice is particularly susceptible to As uptake due to high mobility of As^{III}. Consequently, As is readily taken up by rice roots and accumulates in grain, increasing the risk of As consumption by humans (Meharg and Rahman, 2003; Zhu et al., 2008; Liu et al., 2004a).

There is an urgent need to develop and study mitigation strategies that reduce As accumulation in rice. (Li R et al., 2009). Water management in rice fields has proven to be an effective approach in controlling arsenic bioavailability as it greatly influences the redox conditions of paddy soils by increasing the oxygen content that favors the predominance of As^{V} species (Somenahally et al., 2011; Das et al., 2016). Intermittent irrigation (II) in rice paddies has been a topic of increasing interest, where rice fields are subjected to intermittent cycles of saturated and unsaturated conditions as irrigation is interrupted and water is allowed to subside until the soil reaches a certain moisture level, after which the field is reflooded (Carrijo et al., 2017).

There are three primary factors to consider when establishing water management treatments for rice paddies: number of dry-downs or cycles of interrupted irrigation, severity of dry-down or number of dry days (i.e., soil moisture content), and timing when the treatment will be applied. The study of the optimal conditions for intermittent irrigation is crucial to reduce grain As contamination with minimal to no impact on yield.

Several studies have revealed that II is an effective solution for reducing methane gas emissions, As concentrations in grains, and increasing water-use efficiency. However, it has been found that under certain conditions yields are reduced and cadmium (Cd) accumulation increased (Bouman and Tuong, 2001; Linquist et al., 2015; Carrijo et al., 2017). Under anoxic conditions, Cd exists in soil as cadmium sulfide (CdS), which has low solubility and is not readily available for plant uptake as Cd concentrations in the soil solution are very low. However, following oxidation of rice fields during a dry-down period, Cd is oxidized to $CdSO_4$, which is soluble, readily enters the soil solution, increases its mobility and subsequent plant uptake.

Furthermore, rice, as many other aquatic plants, possesses a porous tissue (i.e., aerenchyma) that transports oxygen to the roots, creating an oxidized rhizosphere. In a flooded paddy soil, the iron (Fe) present in soil minerals undergoes reductive dissolution and the highly soluble Fe^{II} species enters the soil solution. With mobilization to the rice rhizosphere, Fe^{II} is oxidized and precipitates on the root surface of rice plants as a consequence of the oxygenated rhizosphere, forming a heterogeneous mineral coating of iron oxides and hydroxides known as iron plaque (Amaral et al., 2016; Liu et al., 2005; Steinberg et al., 1994; Mei et al., 2009)

The Fe^{III}-oxides and hydroxides in soil and root plaque have a very strong binding affinity for As (Liu et al., 2004 a, b), and oxidation of arsenite to arsenate is thermodynamically favorable (Otte et al., 1991), thus acting as a barrier for As uptake and due to favorable bonding with Fe plaque. Moreover, this buffer effect may depend on the amount of Fe plaque on the root surface (Hu et al., 2007) and its mineral composition, which can be impacted by various factors, including shifting redox conditions induced by water management practices. Soil redox potential (Eh) can be used as an indicator of changes in chemical speciation occurring under II treatments in rice paddy systems. The oxic and anoxic fluctuations directly influence the transformations of redox-active elements such as Fe and As in rhizosphere and bulk soil.

The greatest challenge of applying water management practices in rice paddies is to optimize parameters that balance the advantages and disadvantages. An ideal treatment regime would decrease As concentrations in grain, decrease methane gas emissions, increase water use efficiency, whilst maintaining low Cd concentrations in grain, sustaining yields, and would be suitable for large-scale farming applications.

Previous field-scale rice paddy water management trials have been executed in California, involving several dry-down treatments of varying severity during 2015 and 2016 (Carrijo et al., 2018; Li et al., 2019). These studies demonstrated that intermittent irrigation is a suitable strategy for decreasing As accumulation in rice grain and, under certain treatments, yields can be maintained. However, the overall purpose of investigating different treatments via multiple field-scale trials is to synthesize information about these irrigation strategies and develop management guidelines that are suitable for growers at a commercial tier. A single dry-down may be easier to implement and thus more suitable to reproduce at field-scale, however, at present, we have insufficient information about the effects of single dry-down treatments on As uptake to provide clear and substantiated guidelines to growers.

The major rice producers in the world are China and India (100,000 – 200,000 million tons annually) followed by Indonesia and Bangladesh (20,000 – 50,000 million tons annually) (FAO, 1998). The primary issue concerning these rice growing regions is the high levels of arsenic in soil and groundwater. Soils in Southeast Asia have been reported to reach up to 111 mg kg^{-1} of As and groundwater up to 3200 μg L^{-1} (McCarty et al., 2011; Afzal et al., 2018). Even though much of the As is geogenic in origin, the contamination of rice growing regions is a serious threat to human health. In the US, about 85% of rice is locally produced and the rest is imported from Asia. Rice in the US is mainly produced in the southern states (Arkansas, California and Louisiana) with California (CA) producing about 20% of US rice (TatahMentan et al., 2020). Although As concentrations of soil in CA represent only a fraction of the As in Southeast Asian soils, the importance of investigations on As uptake cannot be underestimated, as there is a global need to integrate strategies to mitigate accumulation of As in grain, particularly for rice that may be produced for infant cereal since the US Food and Drug Administration (US FDA) recommended action level is low, 0.1 mg kg^{-1} (US FDA, 2020). While CA soils and groundwater have much lower As concentrations that in other parts of the world and may not be a perfect representation of processes of As accumulation in other areas, these studies still contribute valuable information to the knowledge base of As fate in rice systems.

In addition to considering II impacts on As and Cd levels in rice grains, it is also important to recognize that rice is a major part of the daily diet of people around the world, providing essential nutrients (e.g., Ca, Mg, Fe, Zn, P, K, Mn) and more than 20% of the caloric intake per day for over 3.5 billion people (Amanullah and Fahad, 2017; TatahMentan et al., 2020). Several factors involved in rice growth may limit the bioavailability of these elements, thus, it is imperative to analyze the impacts of varied II treatments on the nutritional value of rice grains.

The overarching objective of this study is to collect critical data for optimizing water management of rice paddies, which can be used in developing guidelines for growers. The specific objectives are to 1) analyze As content in rice plants over the course of two growing seasons, comparing single dry-down II treatments of varying timing and severities; 2) analyze contaminant (As, Cd) and nutrient (P, K, Zn) concentrations in rice grains over a four year period of II; 3) understand how changes in redox potential may indicate trends in the formation of iron plaque and As sequestration in the roots and rhizosphere; and 4) refine and improve recommendations for rice paddy water management methods.

2. Methods

2.1. Study site and experimental design

This study was conducted at the California Cooperative Rice Research Foundation Rice Experiment Station (39°27'47" N, 121°43'35" W) in Biggs, California, during the summer growing seasons of 2017 and 2018. The experiment consisted of 12 plots that were comprised of 0.2 ha basins with treatments laid out in a completely randomized design with three replications. These are the same plots that were investigated in prior years and published in Carrijo et al., 2018, and Li et al., 2019.

The soil at the site is a Vertisol (i.e., fine, smectitic, thermic, Xeric Epiaquerts and Duraquerts), with a soil particle size distribution 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; Carrijo et al., 2018). The site has a Mediterranean climate with average temperatures of 25.1 ˚C and 23.4 ˚C for summer season 2017 and 2018, respectively (May-October), and an average precipitation of 3.68 mm and 1.86 mm for summer 2017 and 2018, respectively. The mean concentrations of As and Cd in soil for 2017 and 2018 were 3.84 mg kg^{-1} and 0.2 mg kg^{-1} respectively, and the As level in irrigation water was 0.39 ng g^{-1}.

For both years, three drying treatments were established based on the dry-down severity, high (HS), medium (MS), and low severity (LS), and continuously flooded (CF) as a control, each with 3 replicates. The CF treatment was flooded from sowing and drained about 3 weeks before harvest, while the other treatments were drained and reflooded after they had reached a volumetric water content of approximately 40% for LS, 35% for MS, and 25% for HS. In 2017, treatments were drained on different dates before the panicle initiation plant growth stage and reflooded on the same date after they had reached the desired soil moisture content. For 2018, the drain treatment was separated, with LS and MS drained before panicle initiation and HS after. Table 1.1 describes the timeline of field management for both growing seasons.

	Event	**Date**
2017		
	Sowing and flooding	30-May
	HS drain	7-Jul
	MS drain	10-Jul
	LS drain	13-Jul
	All treatments reflood	18-Jul
	All plots drain	17-Sep
	Harvest	16-Oct
2018		
	Sowing and flooding	25-May
	MS drain	1-Jul
	LS drain	8-Jul
	HS drain	9-Jul
	MS reflood	12-Jul
	LS reflood	13-Jul
	HS reflood	23-Jul
	All plots drain	19-Sep
	Harvest	12-Oct

Table 1.1. Sampling dates and irrigation treatments for 2017 and 2018 growing seasons.

2.2. Sample collection and preparation

Three locations from each plot were randomly selected to sample throughout each growing season. Eh and pH measurements were obtained by inserting a probe ~10 cm into the flooded topsoil until the reading was stable. Probes were calibrated on each sampling date.

At each sampling point, throughout the growing season, 15 plants were sampled from each plot. Roots were rinsed immediately with paddy water to remove soil adhered to roots. Plants were stored in a plastic bag and transported at 4 ˚C. Subsequently, shoots were separated from roots and roots were washed thoroughly with deionized (DI) water to remove any remaining soil. Plant biomass was dried at 60 ˚C for 48 hours, cut into 2 cm sections, and a subsample was ground and sieved at 0.5 mm for storage.

For the plants sampled at harvest, panicles were separated, paddy grain was de-husked to obtain brown rice, which was consecutively de-branned to obtain white rice. To remove moisture, grains were dried for 48 hours at 60 ˚C, ground, sieved at 0.5 mm, and stored in airtight containers.

2.3. Iron plaque extraction

Dry roots were weighed, 0.5 g, in 50 mL falcon tubes and 0.5 g of sodium dithionite powder was added, following 40 mL of a solution containing 0.03 mol L^{-1} of sodium citrate and 0.125 mol L^{-1} of sodium bicarbonate. The sealed tubes were mixed for one hour on a horizontal shaker and filtered with 25 µm cellulose filter paper. Roots were rinsed 5 times with DI water. The extract was collected, acidified, and stored at 4 ˚C before analysis. Roots after plaque extractions were dried at 60 ˚C for 48 hours, following grinding and sieving to 0.5 mm for analysis and storage. Root weight was recorded before and after plaque extraction.

2.4. Elemental analysis

For analysis of total As and other elements (Cd, Fe, P, K), powder plant tissue was digested in 70% nitric acid at 150 ˚C until all liquid had evaporated. The residue was re-dissolved in 10 mL of 0.28 M nitric acid with gentle stirring once the tubes had cooled down. The mix was then passed through 0.45 μm filters and diluted for analysis at 5-fold for grain and 20-fold for roots and shoots. Root plaque sodium DCB (dithionite-citrate-bicarbonate) extracts were diluted at 100-fold for analysis.

The solutions from plant tissue digestion and iron plaque extractions were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) Agilent 7900 with germanium and scandium internal standards. For grain samples, NIST standard reference material 1568b (rice flour) was used.

Grain As speciation concentrations were obtained by digesting powdered grain in 0.28 M nitric acid for 90 min at 95 ˚C followed by centrifugation of the digestion product (6048 RCF), dilution of supernatant, and pH adjustment to 9.8. The samples were analyzed using high performance liquid chromatography (HPLC) 1200 series, Agilent Technologies, coupled with ICP-MS 7900. The HPLC column was an anion-exchange Hamilton PRPX100, with an isocratic mobile phase of 10 mmol L^{-1} ammonium phosphate dibasic (pH 8.25).

2.5. Statistical analysis

All treatment effects and differences between sampling dates were assessed using two-way analysis of variance (ANOVA). All effects with p-values < 0.05 were considered significant. P-values are presented throughout, but where significance is discussed, Tukey's multiple comparison tests were conducted.

3. Results

3.1. Redox potential and pH

Following dry-down events, the redox potential (Eh) increased in both years (Figure 1.1) and was greatest in the high severity treatments, indicating oxidizing conditions in the soil. The variation between sampling dates in 2017 is significant with a p-value < 0.001, with Eh increasing from ~110 to ~340 mV. The p-value for the comparison amongst treatments for 2017 and between sampling dates for 2018 falls between 0.05 and 0.1, indicating some difference amongst redox potentials. There is no significant variation between treatments for the 2018 season (p-value > 0.1).

During the 2018 season, the pH increased from June to September by an average of 1.5 units from pH 6 to pH 7.5 for all treatments (See Appendix 1.1). The differences in pH amongst treatments and sampling dates were statistically significant (p-value < 0.001). The CF treatment was always higher than all II treatments for all sampling dates.

3.2. As and Cd in rice grain

Arsenic concentrations in grain decreased with II treatments, with lower concentrations for treatments of longer dry-down periods (Figure 1.2). Total arsenic (*T*As) concentrations in brown rice were higher than white rice (about 2-fold) due to accumulation of As in the bran. Although II treatments were effective in reducing As uptake into rice grains, cadmium concentrations follow an inverse trend where the dry-downs of higher severity increased Cd content in grain (Figure 1.3).

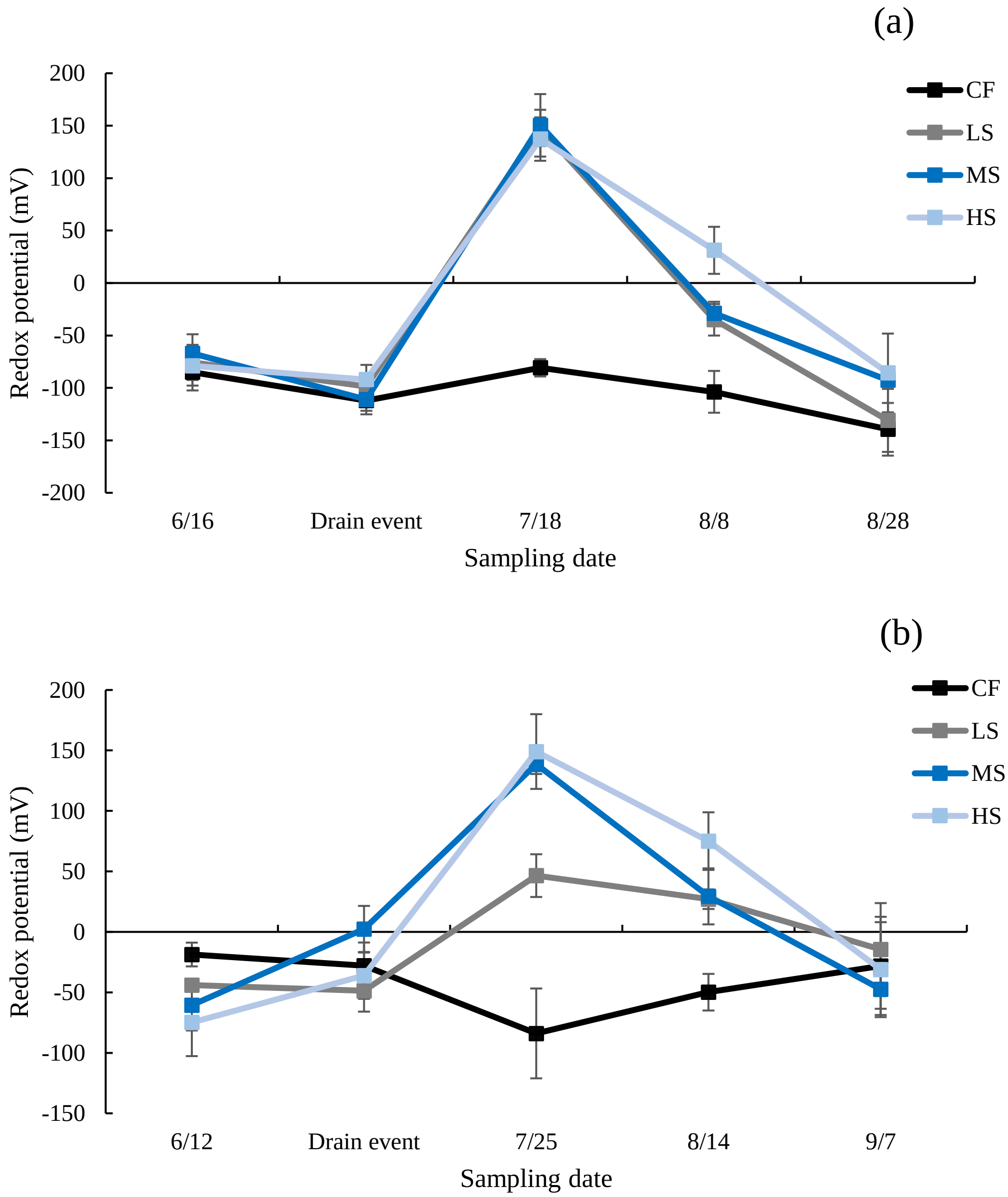

Figure 1.1. Soil redox potential (mV) throughout the 2017 (a) and 2018 (b) growing seasons.

Examining our results on the speciation of As in grain, we observed organic As was present only as DMA and inorganic arsenic (*i*As) in the reduced form as As^{III}. Additionally, MS and HS treatments did not accumulate any DMA. Comparing LS to CF treatment, DMA decreased ~50% for brown and white rice in both years. HS treatments decreased *i*As in grain (compared to CF) by 10.8 and 25.2% for brown grain and 29.2 and 28.4% for white grain in 2017 and 2018, respectively. Cd increased with II treatments 57.5 and 65.9% in brown grain and 71 and 77.3% in white grain in 2017 and 2018, respectively. The interaction amongst treatments for all grain was statistically significant with p-value < 0.05 for As and Cd concentrations.

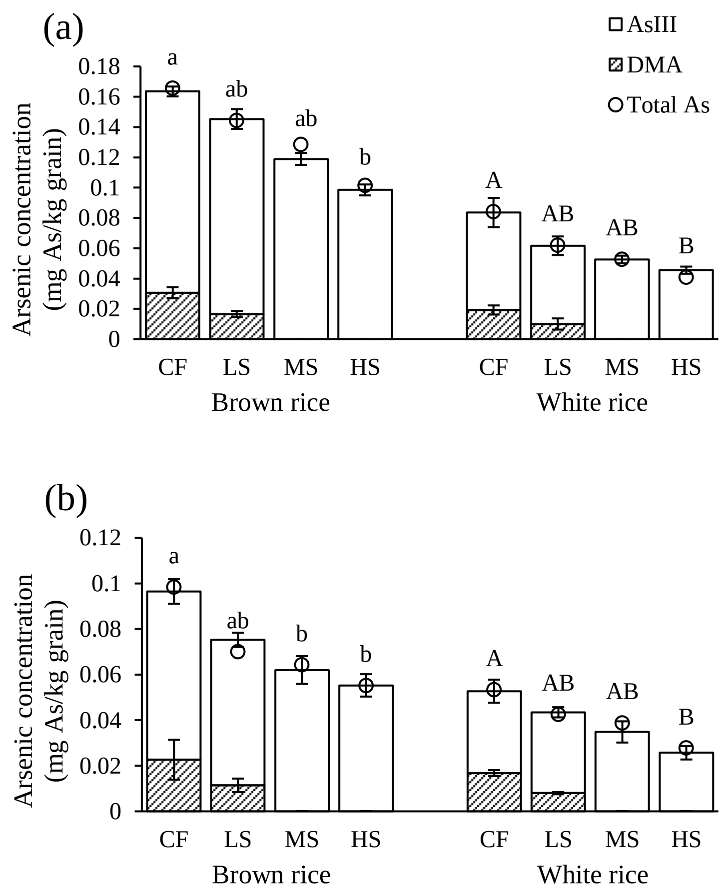

Figure 1.2. Speciation and total arsenic concentrations in white and brown grain for 2017 (a) and 2018 (b).

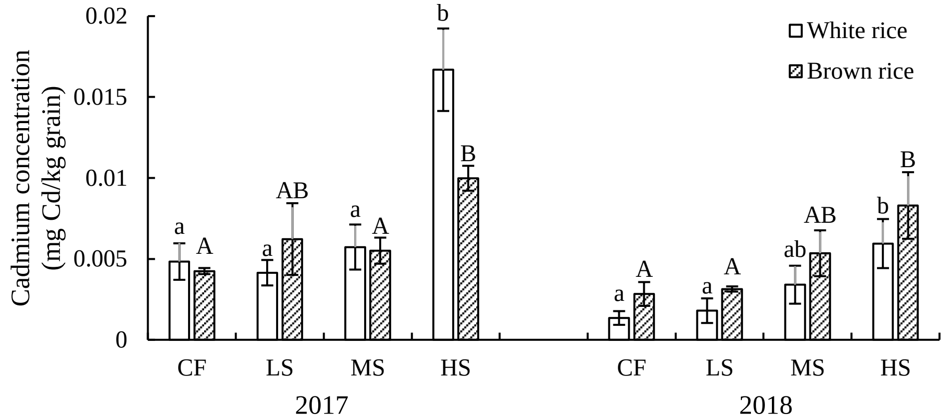

Figure 1.3. Cadmium concentrations in white and brown grain for 2017 and 2018.

3.3. As concentrations in straw and root

As levels in rice straw over the 2017 and 2018 growing seasons are shown in Appendix 1.2. The same trend of As grain concentrations was observed, *T*As concentrations decreased as drying severity increased for all samples following the dry-down period. For shoots at harvest, *T*As concentrations decreased with HS treatment 1.9 and 1.6-fold for 2017 and 2018, respectively, in comparison to the CF treatment. All interactions between treatments and amongst sampling dates for shoots in both years were significant (p-value < 0.01).

The *T*As concentrations for roots with plaque were on average 25-fold higher than shoot As. Roots sampled during 2017 had higher levels of *T*As than those from 2018 (See Appendix 1.3). Overall, *T*As content decreased after dry-down, with higher concentrations in CF treatments. *T*As concentrations in roots at harvest increased for all treatments. Root samples from 2017 show significant interaction amongst sampling dates (p-value < 0.001) but not between treatments, whereas 2018 samples show variation amongst treatments (p-value < 0.001), but not between sampling dates.

3.4. Root plaque analysis

The amount of Fe plaque extracted via DCB solution was 2.3% (percentage by weight) lower for the HS treatments in comparison with CF treatments. About 30 days after dry-down periods, the amount of extractable Fe plaque nearly doubled for all treatments, explaining the statistical significance of the comparisons between sampling dates (p-value < 0.05).

As concentrations of DCB extracted Fe plaque increased during the middle of the season after panicle initiation (7/18/2017, 7/25/2018) and decreased 2.5-fold after heading (p-value < 0.05). We observed the same trend for both growing seasons (Figure 1.4). The differences in As concentration amongst treatments are significant (p-value < 0.05), which may be explained by the higher amount of As accumulated in CF treatment for all dates in comparison to II treatments.

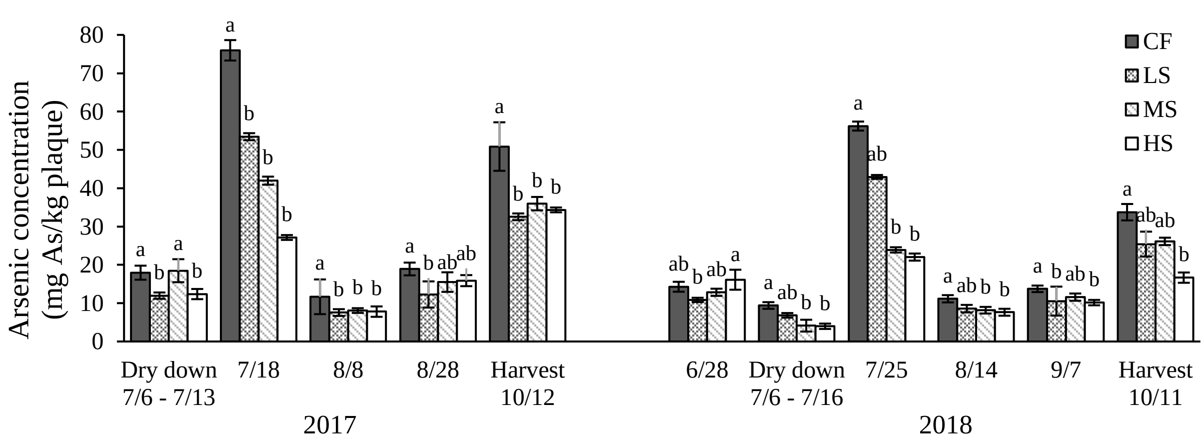

Figure 1.4. Arsenic concentrations in DCB extracted root plaque for both years.

3.5. Nutrient concentrations in grain

Overall, the concentration of Fe, P, and K in white grain increased by an average of 21.9% with HS II treatments for all years, in comparison to CF treatments; and increased by 10.5% in brown grain. Concentrations of these elements are presented in Table 1.2. No significant difference (p-value > 0.05) is found when comparing each element amongst all four years.

Fe concentrations were lowest in both white and brown grain during 2018, ranging from 2 to 2.4 mg kg-1, while the year with the highest Fe concentrations in white grain was 2016 (up to 24.8 mg kg-1) and for brown grain in 2015 (up to 49.7 mg kg-1). Potassium was found in a smaller range, between 0.6 and 1.3 mg kg-1 in white grain, with concentrations being highest for treatments from 2018. In brown grain, K ranged between 2.2 and 3.2 mg kg-1 and was found in higher concentrations in 2017. Lastly, phosphorus ranged between 0.74 and 1.41 mg kg-1 in white grain and was also highest during 2018, while brown grain P concentrations were found between 2.2 and 3.34 mg kg-1 with the highest concentrations in samples of the 2017 season.

		Phosphorus (P)		Potassium (K)		Iron (Fe)	
Year	Treatment	White grain	Brown grain	White grain	Brown grain	White grain	Brown grain
2015	CF	0.92 ± 0.05	2.88 ± 0.06	0.72 ± 0.05	2.58 ± 0.04	11.60 ± 2.69	45.87 ± 13.77
	MS	0.93 ± 0.08	2.75 ± 0.09	0.76 ± 0.08	2.53 ± 0.06	13.48 ± 2.56	52.78 ± 15.05
	HS	0.97 ± 0.07	2.71 ± 0.23	0.79 ± 0.09	2.48 ± 0.18	14.92 ± 1.99	49.65 ± 13.19
2016	CF	0.83 ± 0.11	2.27 ± 0.26	0.70 ± 0.09	2.15 ± 0.31	19.25 ± 1.84	16.15 ± 1.43
	LS	0.84 ± 0.11	2.48 ± 0.11	0.70 ± 0.12	2.41 ± 0.11	15.15 ± 2.47	15.51 ± 1.47
	MS	0.89 ± 0.19	2.68 ± 0.40	0.74 ± 0.20	2.57 ± 0.40	24.84 ± 6.22	15.87 ± 1.18
	HS	1.17 ± 0.14	2.73 ± 0.39	0.97 ± 0.14	2.53 ± 0.38	21.74 ± 4.64	19.10 ± 4.56
2017	CF	0.93 ± 0.25	3.03 ± 0.42	0.76 ± 0.24	2.69 ± 0.60	9.98 ± 2.70	29.90 ± 5.68
	LS	0.96 ± 0.40	3.31 ± 0.38	0.79 ± 0.38	3.22 ± 0.09	8.67 ± 1.71	26.88 ± 7.12
	MS	0.74 ± 0.08	3.22 ± 0.24	0.57 ± 0.08	2.95 ± 0.29	6.39 ± 1.72	30.23 ± 2.98
	HS	1.10 ± 0.41	3.34 ± 0.12	0.88 ± 0.37	3.08 ± 0.12	9.36 ± 2.51	31.42 ± 4.35
2018	CF	1.17 ± 0.09	2.20 ± 0.15	1.14 ± 0.04	2.23 ± 0.08	1.95 ± 0.35	8.06 ± 1.03
	LS	1.37 ± 0.08	2.21 ± 0.20	1.22 ± 0.06	2.30 ± 0.05	2.32 ± 0.41	8.52 ± 0.71
	MS	1.30 ± 0.14	2.21 ± 0.19	1.20 ± 0.07	2.29 ± 0.07	2.09 ± 0.21	7.86 ± 0.92
	HS	1.41 ± 0.24	2.37 ± 0.13	1.27 ± 0.18	2.47 ± 0.07	2.40 ± 0.80	8.56 ± 0.56

Table 1.2. White and brown grain nutrient concentrations (mg kg^{-1}) for 2015, 2016, 2017, and 2018.

4. Discussion

With II treatments, flooding and drying events induced redox fluctuations. During drying events, diffusion of atmospheric oxygen into the anoxic soil results in a rapid increase in redox potential (Eh), with increased drying time leading to more oxic conditions. These redox fluctuations directly impact the speciation of many elements in the soil, including As and Fe, which are critically important for this study.

As expected, II treatments reduced As accumulation in plant tissue and grain. The high severity dry-down treatments were the most effective at reducing As concentrations in plant biomass. Several studies have found that II is an effective method for reducing As accumulation in rice (Linquist et al., 2015; Das et al., 2016; Carrijo et al., 2018; Li et al., 2019), but only a few have measured soil redox potential and evaluated field-scale temporal data that predicts the changes in redox active species.

In 2018, the pH increased over the growing season (See appendix 1.1), with a more alkaline pH observed for the CF treatment in comparison to II treatments. Higher pH increases As^V solubility and can lead to As release into the soil solution and increase its bioavailability for plant uptake (Masscheleyn et al., 1991; Zhu and Merkel, 2001). When paddy soils are initially flooded, the pH drops as CO_2 (present in aerated soils) reacts with water to form carbonic acid. However, reduction processes that occur in soils lead to a net consumption of protons and an increase in pH (Ding et al., 2019).

To help predict the behavior of Fe and As throughout a growing season, we can identify the changes in soil parameters via a Pourbaix (or Eh-pH) diagram. Figure 1.5 shows speciation of As and Fe as a function of Eh and pH. The differences between CF and HS treatments have been labeled in the diagram (green and blue, respectively), and numbered for the sampling dates with number 1 being early in the season (6/12/2018) and 5 towards the end of the season (9/7/2018). Sampling dates and the measured soil parameters can be retrieved from Figure 1.1 and Appendix 1.1. The diagram indicates the presence of reduced forms of As and Fe in CF treatment for all dates, but later in the season, it approaches the Eh-pH conditions, represented by the main diagonal orange (As) and red (Fe) lines, that indicate transformation to the oxidized forms of the elements. In contrast, HS suggests As and Fe are present in the reduced form at the beginning of the season but mid-season, after the dry-down event, the soil conditions suggest oxidation of both species exist for the remainder of the season. Based on the predictions of the diagram, As^{V} remains the dominant species after pH 7 and Eh above 100 mV, as confirmed in Marin et al., 1993 and Suthersan, 2001.

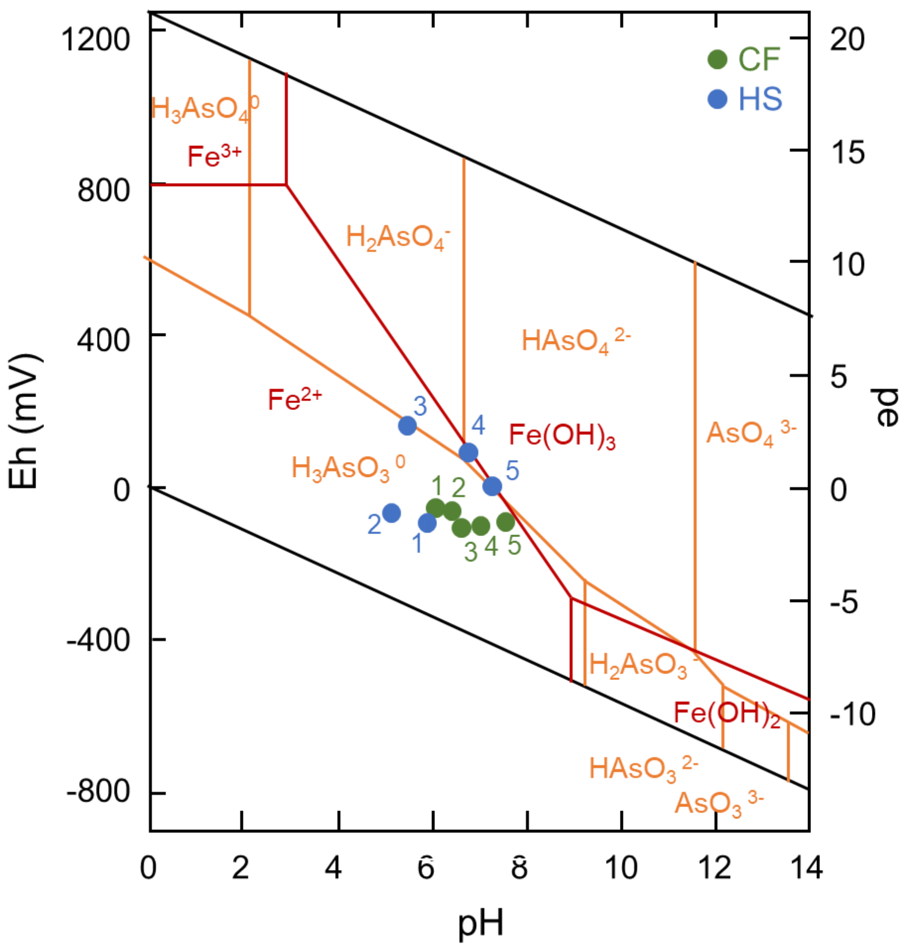

Figure 1.5. Eh-pH diagram (modified from Smedley and Kinniburgh, 2002; and Monhemius, 2017) with 2018 soil parameters identified for CF and HS treatments. Numbers 1 to 5 represent sampling dates in successive order.

Due to certain limitations, redox potential was sampled with a handheld probe which may have disturbed the surface of paddy soil when the probe was introduced for measurement. Notwithstanding, our trend indicates that Eh increased with dry-down treatments, (this was true for all treatments and increased with severity of dry-downs) but it is understood that the anoxic levels in paddy soils could reach below -200 mV at 10-15 cm depth (Hashimoto and Kanke, 2018; Schmidt et al., 2011; Marin et al., 1993).

Our results demonstrate that the HS treatment decreased As concentrations in white grain by 49.5% and 41.3% in brown grain (averaged for both years) and the MS treatment decreased white and brown grain As by 32.2 and 30.4%, respectively. Cd increased for the HS treatment by 393% and 264% for white and brown grain, respectively, but only by 160-185% for the MS treatment. However, it is important to recognize that these elevated Cd concentrations are very low due to low Cd in the soil and water at the experimental site, and do not pose a human health threat.

It is appropriate to evaluate the differences in the water regimes of this study with field-trials from Carrijo et al., 2018 and Li et al., 2019, since they were executed at the same location. These studies examined two dry-down events of varying severity during 2015 and 2016. When compared to CF, their HS treatment reduced *T*As concentration in grain by 54-59% for brown rice and 61-63% for white rice, while MS treatment decreased 41-52% for brown rice and 42-58% for white rice. Li et al., 2019 showed Cd concentrations in grain increased by 322% with HS treatment and 158% with MS. These authors also found that LS treatments were not very effective at reducing As grain concentrations.

Evaluating water management regimes in a larger context by integrating 4 years of data can help us determine what dry-down duration and frequency is ideal for developing suitable management guidelines for commercial-scale rice production. II treatments in both experiments decreased As translocation to rice grains but led to elevated Cd levels. Cadmium is released from CdS as oxygen is introduced into paddy soils during dry-down treatments, oxidizing sulfide and mobilizing Cd (Seyfferth et al., 2019). For this reason, the MS treatment is a more suitable II recommendation for maintaining grain Cd levels low whilst reducing As accumulation; besides, longer drying times do not further decrease As uptake (Li et al., 2019). Another advantage of a less severe drying treatment is to reliably maintain yield by minimizing stress in rice plants during the drying period. In this study, II treatments did not significantly impact yield or yield components, however, it is unrealistic to predict that yield will not be affected by II practices in other locations due to the differences in climate, environmental components, and rice varieties. Combined, these studies demonstrate that moderate dry-downs can be effective at reducing As while minimizing Cd uptake. In developing practices for other locations, these studies serve as a guide to demonstrate how II management strategies can be optimized for yield, human health, and environmental impacts over multiple growing seasons. Of course, there can be conditions where II would have low efficacy and other management strategies should be considered.

Additionally, we did not identify large differences in the reduction of As in grain between one or two dry-down events in a growing season. This demonstrates that applying II treatments of a single dry-down is sufficient to achieve lower As accumulation in rice. This is of great benefit as single dry-down treatments are simple and easy to implement and thus suitable for implementation at the field-scale.

II treatments had a significant impact on grain As speciation. As^{III} was identified as the dominant inorganic species translocated into the grain and organic As, as DMA, was not observed in the more severe treatments (MS and HS) for both white and brown grain, indicating that II reduced organic As accumulation by 100%. For both years, As^{III} accumulation decreased by 25-29% for the HS treatment. Our As speciation results in grain samples was in accordance with Williams et al., 2005, who reported that As in rice grain is present primarily as inorganic As^{III} and As^{V}, with a considerable proportion of organic As, mainly as dimethylarsinic acid (DMA). As^{V} mainly accumulates in grain husks, therefore the only As species identified in brown and white rice are As^{III} and DMA.

Moreover, it has been reported As methylation does not take place within the rice plant, thus the source of methylated As species is most likely the rhizosphere where microbial methylation occurs (Chen et al., 2017). The transformation of As^{III} to DMA is favored under anaerobic conditions in soil and rhizosphere (Abedin et al., 2002; Somenahally et al., 2011), leading to increased DMA uptake.

It is also expected that higher concentrations of As in the soil solution inhibits methylation (Abedin et al 2002), thus overall, and especially in areas with higher concentrations of As in the environment (soil and groundwater), As^{III} is expected to be the predominant species of As in grain, as proven with our data (Figure 1.2); this is the most toxic species to humans and strengthens the necessity to implement strategies to mitigate its uptake and accumulation in rice grain.

As previously stated, the US FDA has proposed an action level for As in infant rice cereals of 0.1 mg kg^{-1} (US FDA, 2020). At a global level, the Joint Food and Agriculture Organization (FAO) and the World Health Organization (WHO) Expert Committee on Food Additives suggested a maximum limit of *i*As of 0.2 mg kg^{-1} for polished rice (FAO and WHO, CXS 193-1995). Therefore, it is important to put in place paddy management strategies of high efficacy in rice growing regions worldwide, even in areas with lower background As levels as these low thresholds can be easily exceeded.

In our samples, all As grain concentrations for white and brown rice were below the FDA's proposed action level, except for 2017 brown grain (Figure 1.2) which did not exceed the FAO/WHO suggested limit, and decreased to ~0.1 mg kg^{-1} after implementation of HS II. Higher concentrations of As in brown rice are expected because grain bran and husk accumulate higher amounts of As (Zhao et al., 2010). Furthermore, the soil at the experimental site had low As concentrations (< 4 mg kg^{-1}) in comparison to other rice growing sites worldwide. As mentioned previously, soils in Southeast Asia can reach As concentrations above 100 mg kg^{-1}. Other studies performed in sites with higher concentrations of As in soil, in comparison to our site, show a similar decrease in As accumulation of grain with water management treatments (Das et al., 2016; Linquist et al., 2015).

For our II treatments, the target volumetric water content (VWC) for LS, MS, and HS was 40, 35, and 25%, respectively. These VWCs are comparable to the field-trials presented by Carrijo et al., 2018 and Li et al., 2019, and aimed to shift soil redox conditions in soil to have substantial impacts on As immobilization without affecting yield (Carrijo et al., 2017). Additionally, the stage of plant growth is important when establishing II practices. It has been previously found that dry-downs early (before panicle initiation), or late (after heading) in the season can have a negative impact to yield and the treatment effectiveness to mitigate As accumulation in grain (Carrijo et al., 2019).

Accumulation of As in rice plant tissue (i.e., straw and roots) can also be affected by II. Our results indicate that roots accumulate the highest amounts of As, followed by straw and finally grain. Throughout the growing season, the trends of As accumulation reveal that the highest levels exist early in the season for straw and later in the season for roots. These differences in accumulation of As in biomass are explained by the detoxification mechanisms that occur when As is first absorbed into root cells (Bakhat et al., 2017), slowing its translocation to rice stems, which consequently decreases As accumulation in shoots early in the season. Regardless of the sampling time, our results show that biomass samples from the CF treatment accumulate more As in shoots and roots, indicating a higher uptake of As by rice plants with uninterrupted flooding due to higher bioavailability of As in the rhizosphere. Similar trends of As accumulation are found in the DCB extracted Fe plaque. The CF treatment accumulated highest concentrations of As in the plaque extracts, while II treatments had less As within the plaque. Additionally, plaque mass is reported as the difference between root mass before and after DCB extraction, which is higher for CF treatment than compared to II (data not shown). This indicates that an increase in soil moisture under anoxic conditions favors iron plaque formation and is consistent with Otte et al., 1991 and Liu et al., 2010. Other studies have found strong collocation of Fe and As on rice root surface (Seyfferth et al., 2010), which explains why a larger amount of iron plaque formed under flooding conditions leads to higher As content in Fe plaque.

As and Cd concentrations in rice grain have been carefully evaluated under different II treatments, however, any reduction in As grain at the expense of rice nutritional value would be unacceptable. Thus, it is crucial to understand how grain nutrient content may be influenced by II. We analyzed macro-nutrient grain concentrations (Fe, P, K) from II treatments of two dry-downs (2015, 2016; samples collected by Carrijo et al., 2018 and Li et al., 2019) and one dry-down (2017, 2018). The data reveal that intermittent irrigation of one or two dry-downs did not negatively impact grain nutrient content. Lower concentrations of Fe present in grain of from 2017 and 2018 compared to the previous years are explained by a change in the site's water supply to surface water with lower Fe concentrations. Moreover, we identified lower concentrations of P in CF treatments compared to all II, with higher concentrations present in HS. In paddy fields, As^{V} acts as a phosphate analog and is taken up by plants via phosphate transporters (Liu et al., 2004). Under flooded conditions, the competition between As^{V} and P in the rhizosphere deters P uptake and higher concentrations of As^{V} are absorbed by the plant. Immobilization of As in soils under oxic conditions reduces this competition and P bioavailability increases, explaining the higher P concentrations in HS treatments. The nutritional value of rice grain is not typically considered when evaluating the impacts of water management treatments on As uptake, water use, and greenhouse gas emissions. However, potential changes in the grain nutritional content should not be ignored, as rice is a staple food for over half of the world's population and a critical source of specific nutrients. In this study, in addition to decreasing As grain content, we reveal that II can increase Fe, P, and K levels in rice grain, providing another potential benefit to II management.

We confirmed that a single dry-down can be a suitable II treatment for minimizing As and Cd concentrations in grain. However, in other rice growing regions different field conditions would exist and II strategies may need to be modified to suit the specific location. To adapt water management regimes to meet specific environmental conditions, a better understanding of the soil and rhizosphere chemistry in rice paddies is crucial. Elucidating the biotic and abiotic changes occurring at the root-soil interface and the mechanisms involved in As immobilization and Fe plaque formation, as well as how these are involved in the broader elemental cycling in rice systems, can bring us a step closer to defining how we can mitigate the ongoing problem of arsenic in rice while addressing human health concerns for one of the most consumed crops worldwide.

5. Conclusions

Single-drain II can be an effective tool for minimizing As accumulation in rice plants without decreasing grain nutrient concentrations and maintaining yields. Cd and As uptake have opposite trends in response to irrigation management practices, longer dry-down treatments decreased As the most but also increased Cd in grain, thus the evaluation of different treatments of varying timing and severity is critical for minimizing both As and Cd grain contamination. Additionally, II practices have a positive impact on rice grain nutritional value, increasing concentrations of Fe, P and K. Although more research is needed to elucidate the specific interactions of As and Fe in the rhizosphere, our study contributes to a greater understanding of these processes and aids in identifying the most suitable II management practices to minimize As accumulation in rice.

CHAPTER 2

Evaluating the impact of rice paddy intermittent irrigation on root plaque and its interactions with arsenic.

Abstract

Arsenic is a naturally occurring metalloid and known carcinogen that is bioavailable in flooded rice paddies and has been a subject of study over the last decades. Under flooded conditions, rice plants transport atmospheric oxygen to the rhizosphere resulting in iron oxide precipitation on the root surface, forming iron plaque composed of mixed mineralogy (e.g., goethite, ferrihydrite) and have a high affinity for binding anions, such as As. Therefore, the potential of root plaque to sequester arsenic and mitigate its uptake and transport to grain will be impacted by mineralogy. Intermittent irrigation (II) has proven to be a suitable technique for reducing arsenic accumulation in grain, however, the mechanisms of arsenic immobilization at the soil-root interface remain unclear. To elucidate the impact of water management treatments in rice cultivation, it is necessary to examine the changes in root plaque chemistry. In this study, we analyzed the mineralogy of iron plaque from rice roots via X-ray diffraction from different II treatments. Additionally, we synthesized goethite and ferrihydrite to observe the binding interactions with arsenate via ATR-FTIR spectroscopy. Our results indicate that ferrihydrite formation is favored with the introduction of oxic conditions during the growing season, and due to its high affinity for arsenate we postulate that it can serve as a sink for As at the root surface and it reduces the bioavailability of As in the rice rhizosphere under II.

1. Introduction

Rice is a staple food for more than half of the world's population and is particularly susceptible to arsenic (As) accumulation (Su et al., 2010). Elevated As uptake occurs because rice is typically grown under flooded conditions where reduced As (arsenite, As^{III}) predominates and has high mobility and toxicity, leading to accumulation in rice grains and increasing the risk for As consumption by humans (Meharg and Rahman, 2003; Zhu et al., 2008; Liu et al., 2004a). Intermittent irrigation (II) is a water management technique that involves intermittent flooding and draining cycles. Several studies reveal that II is an effective solution for reducing As concentrations in grain, methane gas emissions, and increasing water-use efficiency (Bouman and Tuong, 2001; Linquist et al., 2015; Carrijo et al., 2017). However, II treatments need additional study to determine their suitability for widespread application in rice paddies.

Rice and many other aquatic plants transport oxygen to their roots via aerenchyma, creating an oxidized rhizosphere and resulting in the formation of an iron (Fe^{III}) mineral plaque. This process occurs by oxidation of aqueous Fe^{II} present in anoxic soil solution after rice fields are flooded, and the precipitation of iron oxides and hydroxides on the root surface (Amaral et al., 2016; Liu et al., 2005; Steinberg et al., 1994; Weatherill et al., 2016). Iron oxides are extensively studied in numerous disciplines because of their abundance in the environment and high reactivity; they play a vital role in many soil exchange processes. Additionally, Fe plaque formation and composition are affected by environmental factors, such as soil solution composition, pH and redox potential (Eh).

Fe plaque can account for up to 14% of the dry weight of mature rice roots and can serve as a sink for As (Chen et al., 1980). Seyfferth et al., 2017, revealed that Fe and As co-occur on the rice root surface. Other studies indicate that rice root iron plaque primarily retains arsenate (As^{V}) (Liu et al 2004 a, b) and some

As^{III} (Chen et al., 2006; Liu et al., 2005). Methylated As has a lower affinity for sorption onto Fe plaque compared to inorganic As species (*i*As) (Amaral et al., 2016).

In rice plants grown under continuous flooding, 95% of Fe plaque is composed of ferrihydrite ($Fe_2O_3 \cdot 0.5H_2O$) and goethite (α-FeOOH) (Hansel and Fendorf, 2001), and, in lower proportions. lepidocrocite (γ-FeOOH) and siderite ($FeCO_3$) (Seyfferth et al., 2011; Hansel et al., 2002, Xu and Yu, 2013). The mineral composition of iron plaque is a result of specific biogeochemical factors at the root-soil interface, which determine their crystallinity and surface affinity for adsorption of anions and cations (Tripathi et al., 2014; Villacis-Garcia et al., 2015). Ferrihydrite is an amorphous mineral that forms via hydrolysis of ferric iron with a large surface area, high adsorptive capacity and a positive charge under most soil conditions, serving as a sink for many oxyanion elements (e.g., As, P, Se) and organic compounds. Ferrihydrite gradually transforms into more thermodynamically stable and more crystalline Fe^{III}-oxides, such as goethite, via Ostwald ripening. (Das et al., 2011; Villacis-Garcia et al., 2015; Weatherill et al., 2016; Wang et al., 2019).

Moreover, intermittent irrigation treatments create fluctuating oxic and anoxic conditions in soil, which can alter the speciation of redox active elements at the root-soil interface. Iron redox cycling affects the mobility and bioavailability of As due to its high affinity for arsenite and arsenate. Oxidizing conditions in the soil can precipitate Fe^{II} into Fe^{III}-oxides and transform arsenic into As^{V}, while reducing conditions can lead to the dissolution of iron plaque, resulting in the release of Fe^{II}, As^{III} and As^{V} present on root plaque, as well as reduction of As^{V} (Bakhat et al., 2017; Bose and Sharma, 2002).

Intermittent irrigation reduces arsenic mobilization by introducing oxic conditions in the soil during the growing season, oxidizing and immobilizing As in soil. Although it is well understood that with redox fluctuations the chemistry of both Fe and As will be affected, we find a gap in understanding how intermittent irrigation treatments of different timing and severity can impact the formation and mineralogy of iron oxides and thus their binding affinity to As.

In addition, several authors have studied the sorption of As^{III} and As^{V} on iron oxide surfaces with some evidence for specific binding mechanisms (Dixit and Hering, 2003; Ying et al., 2012; Manning et al., 1998; Jain et al., 1999); however, to our knowledge, a distinction of the binding interactions between As^{V} and root plaque iron oxides via *in-situ* experiments to evaluate As sorption on the mineral surface have not been conducted.

In Chapter 1, our research demonstrated that II is a suitable strategy to reduce rice As uptake and translocation into the grain, but the chemical changes occurring in the rhizosphere, the mechanisms for As sequestration and the role of Fe plaque remain unclear. The objective of this study is to identify the changes in root plaque chemistry under intermittent irrigation treatments and to understand the binding interactions of synthesized iron oxide minerals present in root plaque with arsenate (As^{V}) to facilitate an increased understanding of their impact on rice As uptake.

2. Methods

2.1. Sample collection

Details of field experiments and plant tissue sampling can be found in Chapter 1, sections 2.1 and 2.2. Samples in this study are obtained from the 2018 growing season at different stages of plant growth after the drain events occurred from July 1st - 23rd (Table 2.1). Three drying treatments were established based on the severity of the dry-downs: high, medium, and low severity (i.e., HS, MS, LS); and one continuously flooded (CF) as a control.

Samples	**Legend**
July 25th, 2018	S1
August 14th, 2018	S2
September 7th, 2018	S3
October 11th, 2018 (harvest)	S4

Table 2.1. Reference legend for sampling dates from the 2018 growing season.

2.2. Iron plaque extraction

Intact iron plaque was extracted from roots of rice plants sampled at different dates during the 2018 growing season. In the lab, 1 g of dry roots was submerged in 10 mL of 18.2 MΩ cm water and sonicated at 20 kHz for 2 h, maintaining a constant temperature of 25 °C. After sonication roots were filtered and rinsed. The filtrate was then centrifuged at 6048 RCF and the supernatant was discarded. The suspension was freeze-dried and stored in air-tight containers at room temperature.

2.3. X-ray diffraction

About 200 µl of extracted root plaque in suspension was pipetted on glass slides and air-dried for X-ray diffraction (XRD) analysis. Samples were analyzed via a Rigaku Ultima IV X-ray diffractometer, operating at 35 kV, 40 mA with CuKα radiation wavelength of 1.54 Å. Each sample was scanned from 2 to 60 °2Θ. The integrated intensities were determined for all scans using the computer software Jade 9 (Materials Data).

2.4. Scanning electron microscopy

A small amount of freeze-dried root plaque powder was applied on aluminum stubs with conductive carbon tape. Each sample was observed with a Thermo Fisher Quattro S Scanning Electron Microscope equipped with an energy dispersive X-ray analyzer to confirm the elemental composition of the samples. Micrographs were taken to provide a general view of the distinct features of the samples, as well as elemental maps.

2.5. Mineral synthesis

2.5.1. Ferrihydrite

2- Line ferrihydrite was synthesized by dissolving 40 g iron(III) nitrate ($Fe(NO_3)_3 \cdot 9H_2O$) in 500 mL of 18.2 MΩ cm^{-1} water and pH was brought to 7.5 by adding 330 mL of 1 M KOH. The solution was centrifuged and dialyzed in 18.2 MΩ cm water for 48 h with changing the water frequently and measuring electrical conductivity (EC) until the EC remain constant below 1 µS cm^{-1}. The final product was freeze-dried and stored at 4 °C.

2.5.2. Goethite

For the synthesis of goethite, 100 mL of 1 M $Fe(NO_3)_3$ solution was combined with 180 mL of 5 M KOH in a polyethylene flask. The solution was then diluted into 2 L and heated at 70 °C for 60 h, centrifuged at 6048 RCF, washed, freeze-dried and stored at 4 °C.

2.6. ATR-FTIR experiments

Synthesized ferrihydrite and goethite were brought to a suspension of concentrations 1.02 and 2.41 g kg^{-1}, respectively, and sonicated at 45 Hz for 5 min to obtain an even distribution of particles. A ZnSe internal reflection element (IRE) was coated with one of the minerals by depositing 1 mL of the suspension on the surface and subsequently stored in a desiccator to air dry overnight. The crystal was then rinsed with 2 mL of 18.2 MΩ cm water to remove any loose particles and then air-dried. Spectra were obtained at 15, 30, 60, 120 and 240 min after adding 1 mL of a 50 mM sodium arsenate (Na_3AsO_4) solution prepared in 5 mM NaCl (pH 5.99) on the mineral coated IRE.

Background spectra of the IRE, iron minerals and 5 mM NaCl solution were previously collected, as well as spectra of the minerals and 250 mM Na_3AsO_4 (pH 6.05). Before analyses, the FTIR sample chamber was purged for 15 min to reduce CO_2 and water vapor in the collected spectra. Spectral subtractions were conducted using Omnic 9 (Thermo Fisher Scientific, Inc.) to remove the NaCl solution, mineral and unbound As^V contributions to the spectra, revealing bound As^V.

3. Results

3.1. Fe plaque analysis via X-ray diffraction

XRD analysis was conducted to examine the differences in iron plaque formed under different intermittent irrigation treatments. Spectra were compared to the JADE9 software library to identify the mineralogy. Goethite (α-FeOOH), siderite ($FeCO_3$) and lepidocrocite (γ-FeOOH) minerals were identified in the CF sample at harvest, but not ferrihydrite ($Fe_2O_3 \cdot 0.5H_2O$) (see Appendix 2.3).

Table 2.2 shows the identification of ferrihydrite, goethite, lepidocrocite and siderite through the four sampling dates for all treatments. Goethite is observed in all S3 and S4 samples at 21.24 °2Θ. Furthermore, for CF treatment at harvest, two additional goethite peaks are evident at 21.223 and 36.658 °2Θ. Ferrihydrite is observed at 28.494 °2Θ for intermittent irrigation S3 and S4 samples. It is also found in the CF treatment of S2. Lepidocrocite was observed for all treatments of S3 and S4. Some goethite, ferrihydrite and lepidocrocite peaks are found in S1 and S2 but no trend regarding the treatments is identified. Siderite peaks (24.851 °2Θ) are present for all S2, S3 and S4 samples, except for HS treatment at harvest. Siderite is very apparent in S3 for all treatments, showing several peaks (42.319, 42.402, 46.159). Spectral analysis revealed peaks corresponding to quartz (SiO_2) at 20.859 and 26.64° 2Θ in nearly all samples. Iron manganese arsenide ($Fe_{0.02}Mn_{0.98}As$) is observed at 27.82° 2Θ in all samples from all dates.

		Ferrihydrite	**Goethite**	**Lepidocrocite**	**Siderite**
S1	CF				
	LS	+	+	+	+
	MS		+		+
	HS				+
S2	CF	+			+
	LS			+	+
	MS		+	+	+
	HS				+
S3	CF		+	+	+
	LS	+	+	+	+
	MS	+	+	+	+
	HS	+	+	+	+
S4	CF		+	+	+
	LS	+	+	+	+
	MS	+	+	+	+
	HS	+	+	+	

Table 2.2. Identification of iron minerals in intermittent irrigation samples based on XRD peaks.

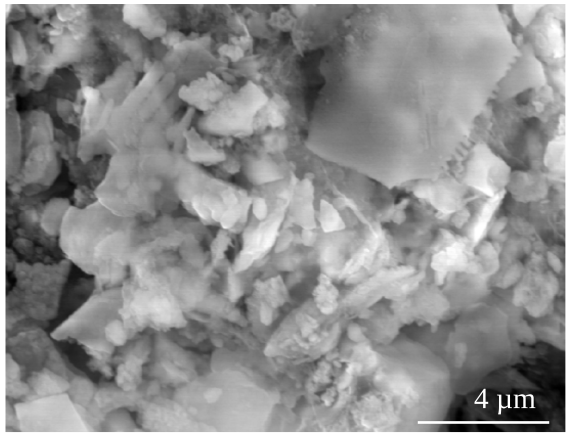

Figure 2.1. Root plaque electron image of continuously flooded treatment at harvest.

3.2. Fe plaque analysis via scanning electron microscopy - energy dispersive X-ray spectroscopy (SEM-EDX)

Images of root plaque were obtained for all treatments of samples from harvest (S4). Figure 2.1 shows an SEM micrograph from the continuously flooded treatment. Elemental maps of the plaque reveal that Fe and Si are not co-located. Arsenic could not be identified as concentrations in plaque samples are below detection limits. Phosphorus is found dispersed in the elemental maps across the entire sample. The SEM-EDX elemental analysis of the plaque, in counts-per-second per electron-volt, show higher amounts of Fe in the CF treatment (859,865 cps/eV) than in the HS treatment (775,988 cps/eV), but lower amounts of P in CF (8,197 cps/eV) than HS (27,239 cps/eV).

3.3. Mineral synthesis

Confirmation of ferrihydrite and goethite synthesis was carried out via XRD, SEM and FTIR spectroscopy. Goethite FTIR spectra show sharp diagnostic peaks at 792 and 890 cm^{-1}, and ferrihydrite is identified by two broad peaks at wavenumbers 475 and 560 cm^{-1} (Schwertmann and Cornell, 2000) (Appendix 2.1). SEM micrographs for goethite show long needle-like crystals and sheets of relatively amorphous particles for ferrihydrite (Appendix 2.2). The elemental analysis of the minerals using EDX spectroscopy is consistent with the chemical composition of goethite and ferrihydrite.

3.4. Mineral ATR-FTIR sorption experiments

Results of the *in-situ* FTIR kinetic binding experiment between goethite and arsenate can be observed in Figure 2.2 and between ferrihydrite and arsenate in Figure 2.3. Contributions from goethite and ferrihydrite in the As-mineral reaction were removed via spectral subtractions to examine changes in the As peaks. Subsequently, aqueous arsenate was subtracted to observe only bound As.

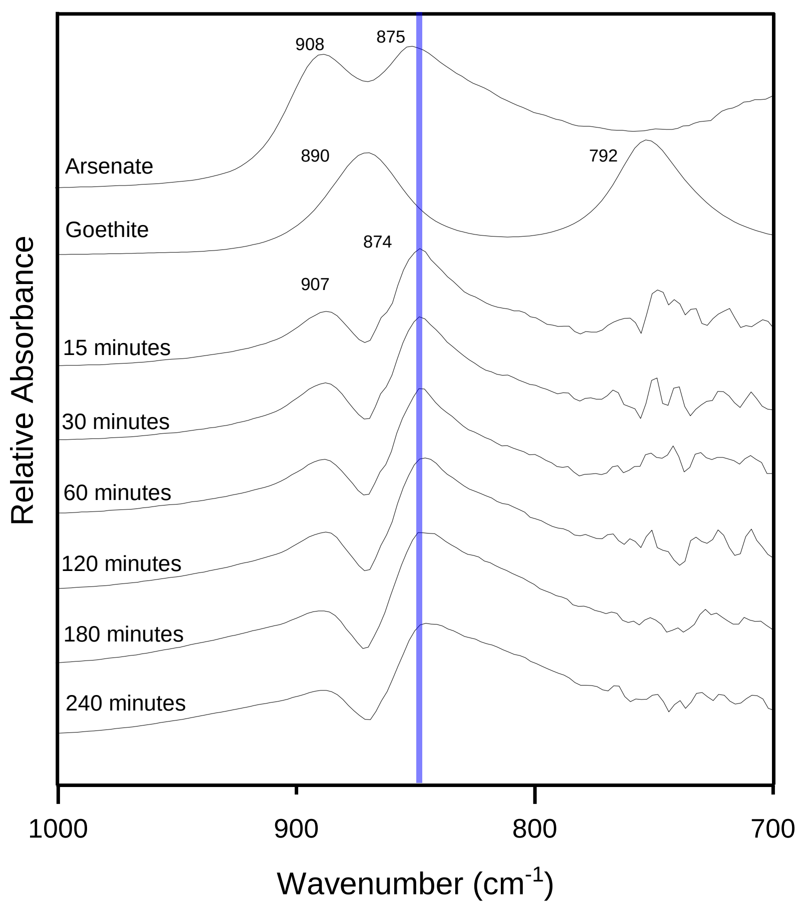

Figure 2.2. ATR-FTIR spectra of arsenate binding to goethite over a time series.

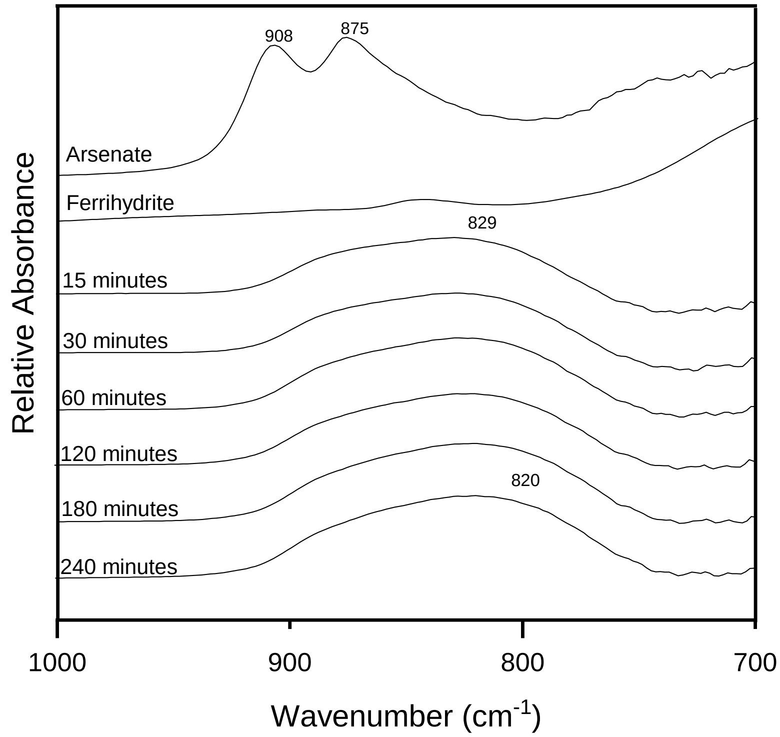

Figure 2.3. ATR-FTIR spectra of arsenate binding to ferrihydrite over a time series.

For goethite, the resulting spectrum after 15 min has two peaks at 907 and 874 cm^{-1}, the latter shifting to 871 cm^{-1} after 240 min and increasing in peak area. The change in peak area from 15 to 240 min was plotted in Figure 2.4, which demonstrates an increase in As sorption on the goethite surface over time. For ferrihydrite, we observe a downshift from 829.4 cm^{-1} at 15 min to 820 cm^{-1} at 240 minutes, as well as peak narrowing, which suggests inner-sphere coordination of As^V to the mineral. The peak area corresponding to arsenate peaks increased by 30% and plateaued at around 120 min indicating sorption and an approach to surface saturation (Figure 2.4).

3.5. Analysis of Fe and As co-localization

Following elemental analysis of DCB extracted plaque performed in Chapter 1, a correlation graph (Figure 2.5) of total Fe and total As demonstrates a positive correlation between As and Fe concentrations. The correlation is greatest for the CF treatment and decreases as the treatments increase in dry-down severity, as observed by the change in slope of the linear regression from 0.0027 to 0.0017.

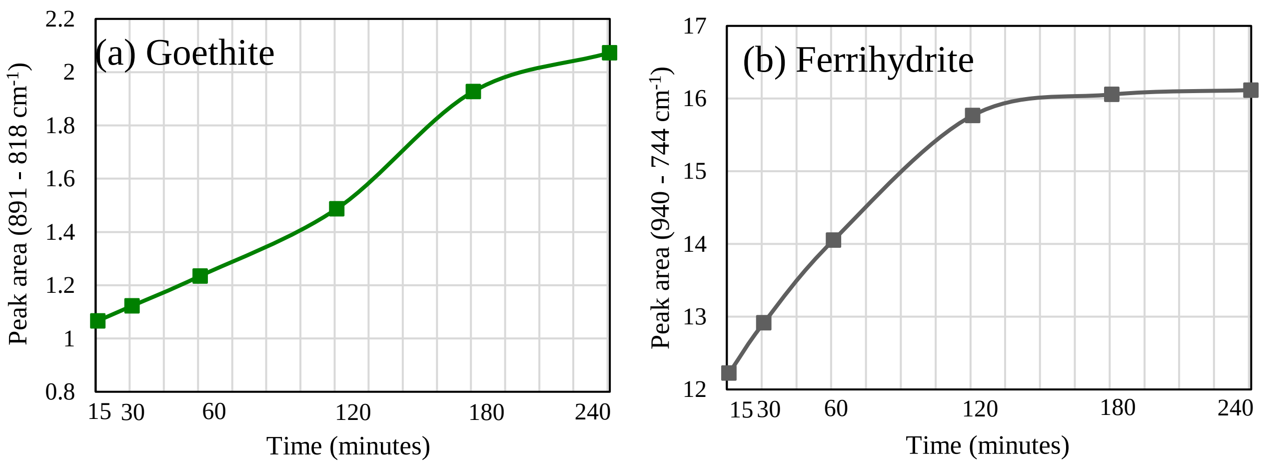

Figure 2.4. FTIR kinetic data show an increase in As^V peak area over time when reacted with a) goethite and b) ferrihydrite. The increased peak area demonstrates increased As^V sorption with increasing reaction time.

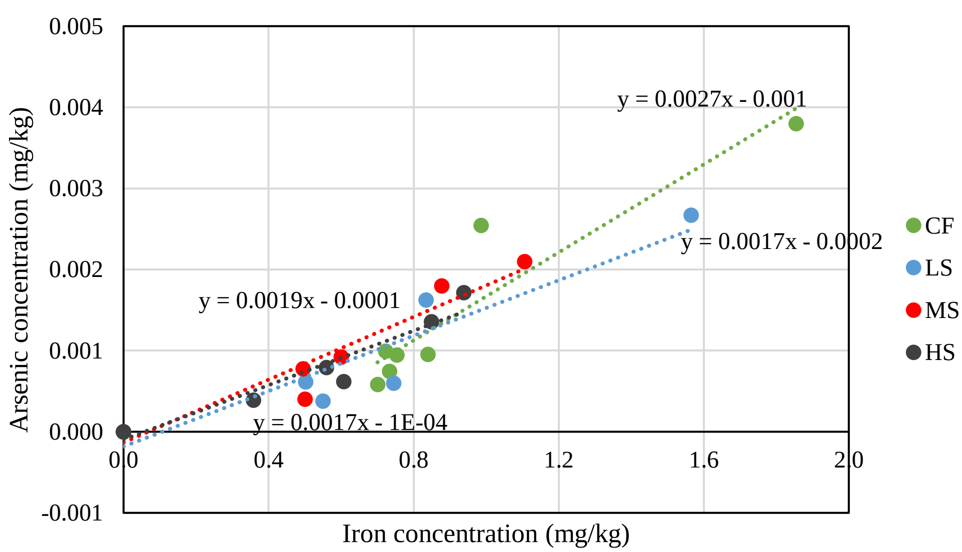

Figure 2.5. Correlation of Fe with total As in DCB extract plotted by treatment.

4. Discussion

The results from this study demonstrate that the mineralogy of rice root plaque is impacted by water management. Ferrihydrite, goethite, lepidocrocite, and siderite were detected in root plaque samples throughout the growing season, which is consistent with prior studies (Blute et al., 2004; Xu and Yu, 2013).

In our XRD results, goethite was observed in all plaque samples at the late growth stages (Table 2.2). Goethite formation typically follows a mineral ripening process where relatively amorphous and soluble minerals are transformed into a more thermodynamically stable and crystalline phase under oxidizing conditions (Das et al., 2011). Additionally, ferrihydrite was first observed in the XRD spectrum corresponding to the CF treatment, as well as in II treatments at the late growth stages. Several factors can influence the mineralogy of rice root plaque, such as pH and soil solution chemistry. As pH decreases from 7 to 5, ferrihydrite is more likely to form (Schwertmann and Thalmann, 1976). In the present study, during the 2018 growing season, soil pH increased from 6 to 7.5, making conditions favorable for ferrihydrite formation during the early stages of the growing season. Additionally, this pH range can increase the retention of arsenate by ferrihydrite than goethite (Jain et al., 1999).

Moreover, Limmer et al., 2018 found that silicon (Si) concentrations during the formation of Fe plaque may play a role in its mineralogy. When the ratio of Si/Fe increases, ferrihydrite is more likely to form rather than lepidocrocite (Schwertmann and Thalmann, 1976). Our SEM results demonstrate that Si and Fe do not co-localize in root plaque for all samples. Higher amounts of Si in HS treatment in comparison to CF were found, indicating greater formation of ferrihydrite on roots of II treatments.

Under flooded conditions, the transport of oxygen via aerenchyma to the rhizosphere drives the oxidation and precipitation of amorphous iron oxides which are subsequently transformed into goethite (Mei et al., 2012; Das et al., 2011). Nevertheless, the identification of ferrihydrite and goethite at different times of the growing season indicates that mineral formation processes are impacted by II, which alters the mineralogy of plaque at different stages of rice growth. Iron plaque formation was studied by Long et al., 2019, revealing that Fe plaque formation occurs progressively; however, it is difficult to identify temporal trends in the mineralogy regarding II treatments when Fe-oxide mineral formation begins, during the early stages of rice growth.

XRD peak quantification and intensity measurements were limited by the presence of quartz (SiO_2) in all samples. Attributed to its highly ordered crystalline structure, absolute intensities of quartz peaks in bulk mineralogy XRD analysis may cause extinction effects and reduce intensities of other mineral peaks, making identification of co-existing minerals more challenging (Snyder, 1992). Additionally, XRD peaks do not often reflect the concentrations of all minerals present in the sample. In some cases, experimental errors or the lattice parameter d (interlayer distance in the Bragg equation $2d\ \mathrm{Sin}(\theta) = n(\lambda)$) will cause the peak positions to vary due to an angle shift. In addition, more crystalline minerals are easier to identify: ferrihydrite being highly amorphous is harder to identify in comparison to a more crystalline mineral such as goethite. For this reason, we focus our discussion on peak identification rather than concentration. Moreover, corresponding XRD peaks from samples collected at the later stages of rice growth, when root plaque mineral composition is less dynamic, are easier to identify in XRD spectra.

Furthermore, Liu et al., 2006 found that ferrihydrite and goethite are the iron minerals present in the highest proportion in rice root plaques; therefore, it is critical to understand the binding interactions of these minerals with arsenic. Although the strong adsorption of As^{V} onto ferrihydrite and goethite has been demonstrated in earlier studies (Foster et al., 1998; Fendorf et al., 1997; Waychunas et al., 1993), there is uncertainty about the binding kinetics, and more studies are needed about how the sorption of arsenate compares between these minerals. Thus, *in-situ* ATR-FTIR experiments were carried out to elucidate binding mechanisms of As^{V} to these Fe-oxides, which are dominant in the root plaque samples from this study. If specific binding mechanisms can be determined it would improve our understanding of the availability of arsenic for plant uptake under different rice paddy water management.

Kinetic analysis results of FTIR spectra for As^{V} reacted with both goethite and ferrihydrite reveal an increase in peak area as a function of time, indicative of As sorption (Figure 2.4). These kinetic binding experiments also exhibit a cap on sorption sites of the surface of ferrihydrite based on the peak area change, showing a quick increase of sorption from 15 to 120 minutes. In contrast, for goethite, arsenate sorption increases slower and begins to plateau around 240 minutes. Additionally, we observe a difference in sorption when comparing the delta peak areas of ferrihydrite compared to goethite, being 8-fold higher for ferrihydrite and deducing that it has an increased ability to adsorb arsenate. It is logical to observe that ferrihydrite binds more As^{V}, as it is a poorly crystalline mineral and its amorphous characteristics are indicative of a greater binding surface and thus can lead to strong affinity for both arsenate and arsenite. These results are in agreement with Huang et al., 2011, which demonstrated higher sorption of *i*As for ferrihydrite than goethite, explained by their differences in specific surface area and microporosity.

Based on the observed aqueous As^{V} diagnostic peaks at 907 and 876 cm^{-1} (pH 6), we identify distinct peak downshifts in all spectra. When As^{V} is reacted with goethite IRE coatings, one peak shifts to 871 cm^{-1} (Figure 2.2). For As^{V} reaction with ferrihydrite, a broad peak appears from 883 to 789 cm^{-1} (Figure 2.3). A downshift in the location of the FTIR peak for dissolved species upon reaction with a mineral surface is commonly attributed to inner-sphere coordination (Hafner and Parikh, 2020; Parikh et al., 2014). The reaction of arsenate with iron oxides has been previously studied via H^{+}/OH^{-}release stoichiometry titration experiments with strong evidence that arsenate is adsorbed on iron oxide surfaces by forming inner-sphere bidentate binuclear complexes (Jain et al., 1999). Inner-sphere binding, particularly bidentate, is substantially stronger than outer-sphere binding and represents a species of Fe that is less bioavailable.

Moreover, prior research has demonstrated that iron and arsenic co-occur on the root surface of rice (Seyfferth et al., 2010; Li et al., 2015), which is consistent with the positive correlation between Fe and As measured in the DCB extracted plaque for all treatments in the current study (Figure 2.5). Furthermore, linear regression shows a decreasing change in slope from CF treatment to MS and HS treatments which is explained by the non-linear sorption of As and the different forms of Fe in plaque (mineralogy), demonstrating that Fe in the CF treatment has a higher affinity for As in comparison with II treatments.

In Chapter 1, our results demonstrated that total grain As concentrations are higher in the CF treatment in comparison to II. In addition, we found that iron plaque in the CF treatment can bind greater amounts of As (Figure 2.5). Overall, more As is present and mobilized in rice plants under continuous flooding. Therefore, we postulate that under II the main mechanism for reducing As accumulation in grain is not directly its sequestration in the rhizosphere but rather the oxidation and immobilization of As in bulk soil which drives the observed sequestration in the rhizosphere. Nevertheless, changes in the mineralogy of root plaque play a key role in reducing the accumulation of As in grain. Elucidating the abiotic changes in Fe and As chemistry that occur in soil due to II facilitates our understanding of the mobilization and bioavailability of As in rice paddies. However, research on the concurrent biotic impacts on As bioavailability and soil health with II is required and this information should be included in a conceptual model for As mobility and bioavailability in rice

5. Conclusions

The changes in oxic and anoxic conditions in soil due to II treatments play a critical role in iron plaque formation on rice roots and the uptake of As into grain. This study provides novel data regarding important mineralogy changes in rice root plaque that contribute to the sequestration of arsenic via adsorption on the surface of ferrihydrite and goethite. Ferrihydrite shows a higher affinity for arsenate adsorption, and it is expected to be present in II samples throughout the growing season. In contrast, goethite binds less arsenate and is likely to be found in greater amounts in the CF treatment samples due to continuous anoxic conditions which favor more stable and crystalline iron oxides. Nevertheless, the impact on As mobility is primarily driven by its immobilization in the soil that decrease their bioavailability in the rhizosphere. Our findings represent an important step forward toward understanding the extent of the impact of intermittent irrigation treatments on the complex As sequestration mechanisms at the soil-root interface and provide insight into the cycling of As and Fe in rice systems.

CHAPTER 3

Evaluating the impact of intermittent irrigation on rhizosphere microorganisms under simulated rice paddy conditions

Abstract

Water management techniques to reduce greenhouse gas emissions and arsenic accumulation in rice grain continue to gain attention. Single drain intermittent irrigation (II) has demonstrated potential for on-farm use at field-scale to achieve these goals. It is important to understand the chemical interactions occurring at the root-soil interface that contribute to the bioavailability of arsenic (As). Although, it is well known that microbes play several critical roles in agroecosystems, little information is known about the impact of bacterial communities with intermittent irrigation and, specifically, their role in As cycling and bioavailability in the rhizosphere. In this study we performed plant growth bioassays to evaluate three single drain treatments that mirrored previous studies conducted at field-scale, with the objective of analyzing microbial 16S rRNA gene variations within the rhizosphere during different stages of rice growth. Our results suggest that the introduction of oxic conditions increases the abundance of aerobic bacteria that contribute to the oxidation and precipitation of Fe in the rhizosphere, and consequently As immobilization. In contrast, under flooded conditions Fe-reducing anaerobic bacteria were dominant. Overall, increasing the severity of II treatments induces a greater shift in the rhizosphere bacterial community of rice. Our study is a step forward in understanding chemical and biological interactions affected by water management treatments in rice systems.

1. Introduction

Rice plants readily take up and accumulate arsenic in the grain when grown in flooded paddy fields, posing a threat to human health (Somenahally et al., 2011). Intermittent irrigation (II) has been a subject of increasing interest due to its efficacy in reducing As accumulation in rice, reducing methane gas emissions, and increasing water use efficiency during rice cultivation (Li et al., 2019; Carrijo et al., 2018).

II is characterized by a distinct cycling of flooded and non-flooded periods that are accompanied by redox fluctuations, which have a significant impact on the fate and form of certain contaminants and nutrients in soil, as well as soil microorganisms (Hare et al 2017; Hu et al., 2015; Takahashi et al., 2004). The cycling of redox active elements in soil is often associated with abiotic factors such as mineral precipitation and reductive dissociation; or biotic factors such as the activity of plant roots and related microorganisms (Mei et al., 2009).

The biogeochemical cycle of As is linked to microbial mediated transformations (e.g., oxidation, reduction, methylation) and influences the mobility, distribution, and availability of As species in the environment. In rice paddies, microorganisms play vital roles in both aerobic and anaerobic soil conditions. During continuously flooded treatments, some anaerobic bacteria can use As^{V} as a terminal electron acceptor in respiration and subsequently reduce it to As^{III}, contributing to greater As bioavailability in the soil solution (Huang et al., 2010; Jia et al., 2014). In aerated soils, some aerobic bacteria can transform As^{III} to less toxic forms, such as As^{V} and methylated As (Hare et al., 2017). Arsenic reducing and oxidizing bacteria often coexist in the rice rhizosphere, and their abundance and activities regulate As speciation, bioavailability, and accumulation in rice paddies (Jia et al., 2014). The relative abundance and activity of As transforming microorganisms are key factors that influence the fate of As in paddy soils, and consequently the bioavailability of As to rice plants (Wang et al., 2019).

Moreover, it is well documented that numerous bacteria species are involved in iron oxidation within the rhizosphere, and thus the microbial community may also play an important role in Fe plaque formation (Steinberg and Coonrod, 1994).

Dissimilatory iron-reducing bacteria use Fe^{III} from iron oxide minerals as a terminal electron acceptor during anaerobic respiration. This reductive dissolution reactions facilitates the release of As from iron oxides, increasing its bioavailability for rice uptake. On the other hand, the oxidized microenvironment created by the oxygen secreted from rice aerenchyma allows iron-oxidizing bacteria to couple the oxidation of Fe^{II} with the reduction of a variety of electron acceptors, promoting the co-precipitation of As with iron oxides (Zecchin et al., 2017; Wang et al., 2019).

Rice systems provide a variety of niches to sustain microbial diversity (Wang et al 2019). Microorganisms are very sensitive to small changes in their environment and can be influenced by a range of biotic and abiotic factors (Fakruddin and Mannan, 2013; Chen et al., 2008). It is thus expected that redox fluctuations caused by II events will affect microbial activity and succession in the rhizosphere.

II is a promising management strategy for reducing As concentrations in grains but needs to be accepted by rice growers for widespread adoption in rice cultivation. Scarcity of information withholds farmers from making well informed decisions. Although growers are typically most concerned about how changing on-farm operations will affect their agronomic systems, there remains a need for mechanistic biotic and abiotic information to explain broad implications of establishing II treatments. At present, the impact of microbial processes on As cycling in the rhizosphere is not well understood. Thus, there is a need for studies that investigate the association between water management treatments, changes in soil microbial communities, and the influence of bacteria in As and Fe cycling in rice systems. Studies that analyze the impact of II on microbial community composition often report treatments with several dry-down events and yield is not aways evaluated (Somenahally et al., 2011; Das et al., 2016; Wang et al., 2019; Zecchin et al., 2017; Chen et al., 2008).

Understanding the changes of microbial populations due to paddy water management regimes can provide information about the role of rhizosphere bacteria in Fe plaque formation and As speciation, bioavailability, and mobility in rice systems. The primary objective of this study was to reproduce rice field conditions in a series of pot-based bioassays with single dry-down II treatments of varying severity and to observe the effect of water management fluctuations in rhizosphere soil bacterial communities throughout the growing season.

2. Methods

2.1. Study site and experimental design

The experimental design aimed to replicate the field conditions from the field-based irrigation management rice growth trials conducted at the California Cooperative Rice Research Foundation Rice Experiment Station (RES) in Biggs, CA during summer 2017 and 2018 (see Chapter 1). The current study was conducted in Escondido, CA (33.185° N, 17.086° W) during summer 2020. The site has a Mediterranean climate with an average temperature of 20.6 °C and average precipitation of 0.813 mm for May - October 2020. Soil from the RES was collected, mixed to homogenize, and placed into 1-gallon pots. The average As and Fe concentrations in the soil were 3.87 mg kg^{-1} and 33.39 g kg^{-1}, respectively.

The three II treatments (replicated from prior field experiments) were high, medium, and low severity (i.e., HS, MS, LS); and one continuously flooded (CF) as a control. Each treatment was assigned to a 62 L plastic bin that contained 6 replicates of 1 gal pots with paddy soil. Rice seeds were planted evenly on the soil surface and the bins were flooded 10 cm above the soil line. Every 5 days water was added to reach the initial water level, pH and redox potential (Eh) were measured, and bins were re-oriented and re-positioned based on a randomized complete block design (RCBD). For the dry-down events, the water from the three II bins was drained and soil moisture was measured on an hourly basis utilizing a Watermark handheld meter and soil moisture sensors set at a depth of 10 to 15 cm. Soil was sampled throughout the season on similar dates from the field experiment presented in Chapter 1. Table 3.1 provides the timeline for sampling, water treatment management, and other events throughout the experiment.

Crop development, management practice, and sampling.	Date	Days after sowing
Sowing and initial flooding	31-May	0
Start of seed germination	3-Jun	3
All treatments soil sampling	25-Jul	55
All intermittent irrigation treatments drain	26-Jul	56
LS reflood and soil sampling	27-Jul	57
MS reflood and soil sampling	29-Jul	59
HS reflood and soil sampling	31-Jul	61
All treatments soil sampling	4-Aug	65
CF flowering	9-Aug	70
All II treatments flowering	19-Aug	80
All treatments soil sampling	29-Aug	90
All treatments soil sampling	6-Oct	128
CF drain	6-Oct	128
LS drain	10-Oct	132
CF harvest	11-Oct	133
MS drain	15-Oct	137
LS harvest	16-Oct	138
MS harvest	19-Oct	141
HS drain	20-Oct	142
HS harvest	23-Oct	145

Table 3.1. Summary of events during pot-based bioassays of rice.

2.2. Sample collection and preparation

Soil was collected using a 1 mL sterile syringe with the end-tip cut off to create a miniature soil probe. Approximately 1 g of soil was obtained at a depth of 5-10 cm at the base of the rice plants (between the roots), and two samples were obtained from each pot. A 0.25 g subsample was separated, and DNA was extracted with the Qiagen DNeasy PowerLyzer PowerSoil Kit.

At harvest, the rice grain was collected, and plants were rinsed to remove soil adhered to the roots. Roots were separated from the shoots and washed thoroughly with DI water to remove any remaining soil. All plant biomass (i.e., grain, shoots, roots) was dried at 65 °C for 24 h. Paddy grain was polished into white grain (de-husked and de-branned) utilizing a rice huller and mill, and each layer was ground, sieved at 0.5 mm, and stored in airtight containers. Roots and shoots were cut into 2 cm sections and a 5 g subsample was ground and sieved at 0.5mm and stored for further analysis.

2.3. Analysis of plant tissue

For analysis of total As in grain, shoots, and roots, as well as total Fe in roots, ground plant tissue was digested in 70% nitric acid at 150 ˚C until all the liquid was evaporated. The residue was re-dissolved in 10 mL of 0.28 M nitric acid with gentle stirring once the tubes had cooled down. The resulting suspensions were passed through 0.45 μm filters and diluted for analysis at 5-fold for grain and 20-fold for roots and shoots. Plant tissue digestion samples were analyzed via inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7900) with germanium and scandium internal standards. For grain samples, NIST standard reference material 1568b (rice flour) was used.

2.4. Microbial sequencing

DNA extractions and library preparation were performed by the UC Davis Host Microbe Systems Biology Core Facility. Primers 341F and 806R were used to amplify the V3-V4 domain of the 16S rRNA using a two-step PCR procedure. Step one was the amplification procedure where the primers contained an Ilumina tag sequence, a variable length spacer, a linker sequence and the 16S target sequence. In step two, each sample was barcoded with a unique forward and reverse barcode combination using forward and reverse primers. The final product was quantified on a Qubit instrument using the Qubit High Sensitivity dsDNA kit (Invitrogen) and individual amplicons were pooled in equal concentrations. The library was quantified via qPCR followed by 300-bp paired-end sequencing using an Illumina MiSeq instrument (Illumina) and taxonomic groups were assigned using the Silva rRNA database.

2.5. Statistical analysis

Data of quantified As and Fe in plant biomass, all treatment effects, and differences between sampling dates were assessed using a two-way analysis of variance (ANOVA). All effects with p-values < 0.05 were considered significant. P-values are presented throughout, but where significance is discussed, Tukey's multiple comparison test was conducted. Relationships between samples from microbial analysis were visualized using principal coordinate analyses (PCoA) obtained based on Bray Curtis dissimilarity metrics.

3. Results

3.1. Soil Eh and pH

Soil pH increased during the growing season from 5.5 to 7 (see Appendix 3.1). Based on an average linear regression for the pH measured at each treatment, CF has a greater increase in pH in comparison to

II treatments. Soil Eh (Figure 1) drops below 0 mV approximately 3 days after sowing. The general trend indicates that redox potential was lower as electrode depth increased, reaching below -400mV. During the dry-down period, Eh increased to 154, 240, and 163 mV for LS, MS, and HS treatments, respectively. Eh dropped rapidly, < -300mV, after II treatments were reflooded. The redox potential at CF treatment was negative (mV) throughout the growing season, and reached its lowest value, -463 mV, 120 days after sowing.

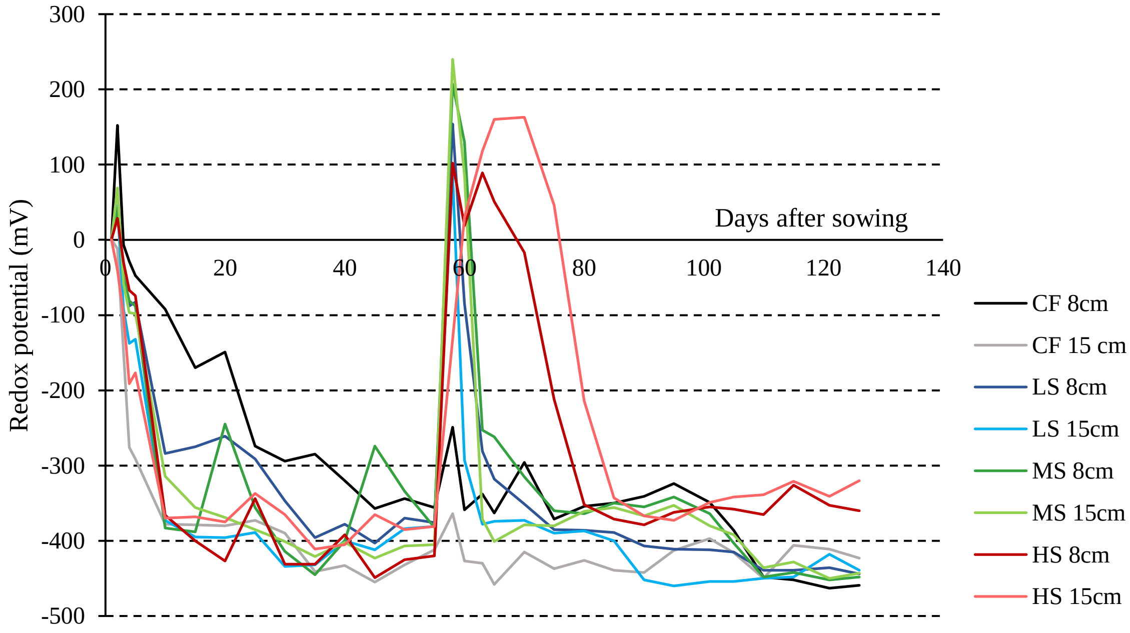

Figure 3.1. Soil Eh throughout the growing season measured at 8 and 15cm depth.

3.2. Soil water potential

The soil water potential (WP) (Figure 3.2) remained at zero while fields were flooded, for all treatments. However, following drain events the WP decreased, after 24 h the LS treatment was -10 kPa, at 83 hours the MS treatment decreased to -72 kPa, and at 132 h the HS treatment was -123 kPa. Following reflooding, the soil WP for all treatments rapidly increased to 0 kPa.

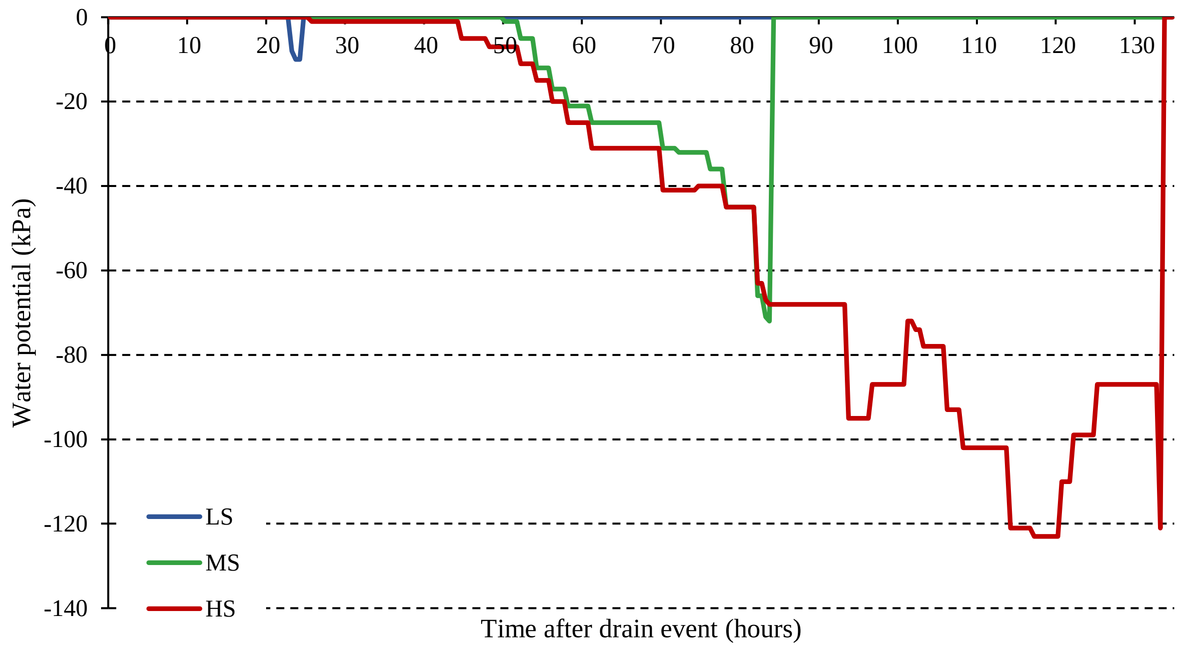

Figure 3.2. Soil water potential during drain events, measured at a 10-15 cm depth.

3.3. Total As in plant tissue

As concentrations in rice grain significantly decreased (p-value < 0.05) from the CF treatment to MS and HS (Figure 3.3). White rice from the CF and HS treatments had mean As concentrations of 0.086 and 0.066 mg kg^{-1}, respectively. The mean As concentration of the bran was 0.417 and 0.282 mg kg^{-1} for the CF and HS treatments, respectively. For the rice husk, mean As concentrations were 0.537 and 0.338 mg kg^{-1} for the CF and HS treatments, respectively.

As concentrations in rice shoots and roots follow the same general trend as the grain, with the highest concentration in the CF treatment and had lower values for LS, MS, and HS (Figure 3.4). The concentration of As in the roots was 23.8, 22.5, 17.6, and 15.8 mg kg^{-1} for CF, LS, MS, and HS, respectively. The concentration of iron in the roots was significantly lower in the II treatments, with As concentrations of 2547.6 mg kg^{-1} in CF and 1190.6 mg kg^{-1} in HS (p-value < 0.05). The roots were analyzed without extracting iron plaque, thus the concentrations of As and Fe represent the total amounts in the roots and Fe plaque combined.

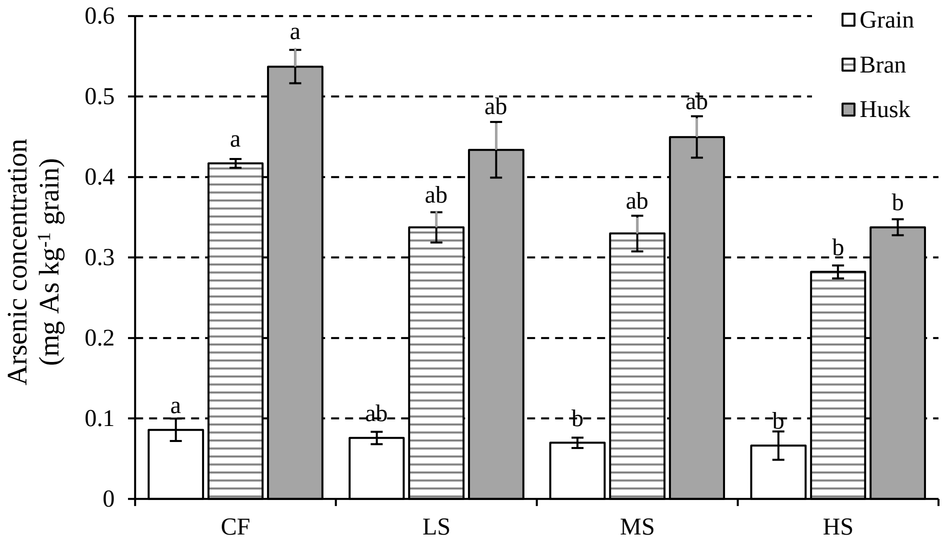

Figure 3.3. Concentrations of As in rice grain at harvest.

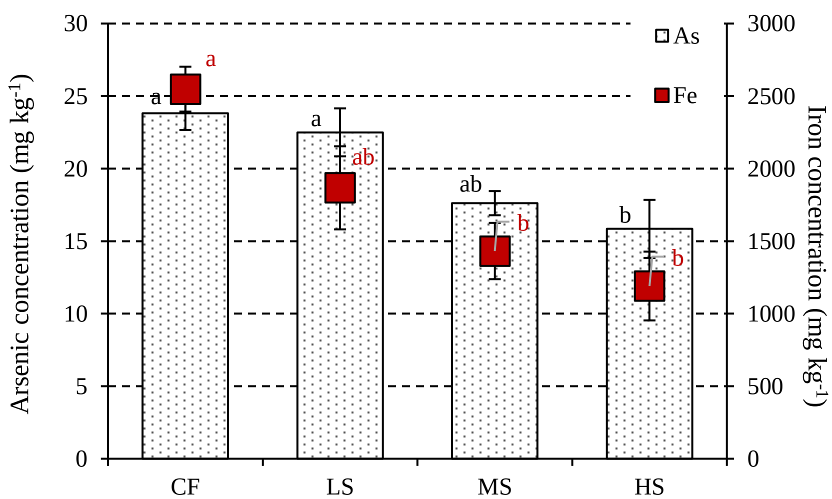

Figure 3.4. Concentrations of As and Fe in rice roots with plaque.

3.4. Microbial abundance and diversity

A total of 1,052,939 confident bacterial 16S rRNA reads were obtained and analyzed. Read classification at a sequencing depth of 18,000 reads revealed about 1100 features per treatment (Appendix 3.2). The highest number of unique sequences were found in the CF treatment. The relative abundances of rhizosphere bacteria were higher for the HS treatment, followed by MS, LS, and CF (24.9, 23.9, 23.3, 20.9 ± 2%, respectively).

In our samples, 52 different phyla were identified with the 15 most abundant comprising 98.2% of the total phyla (Figure 5). Additionally, 155 classes, 353 orders, 546 families, and 942 genera were identified. Soil sampled before sowing had lower percentages of the dominant bacteria, comprising 5.1% of the bacteria.

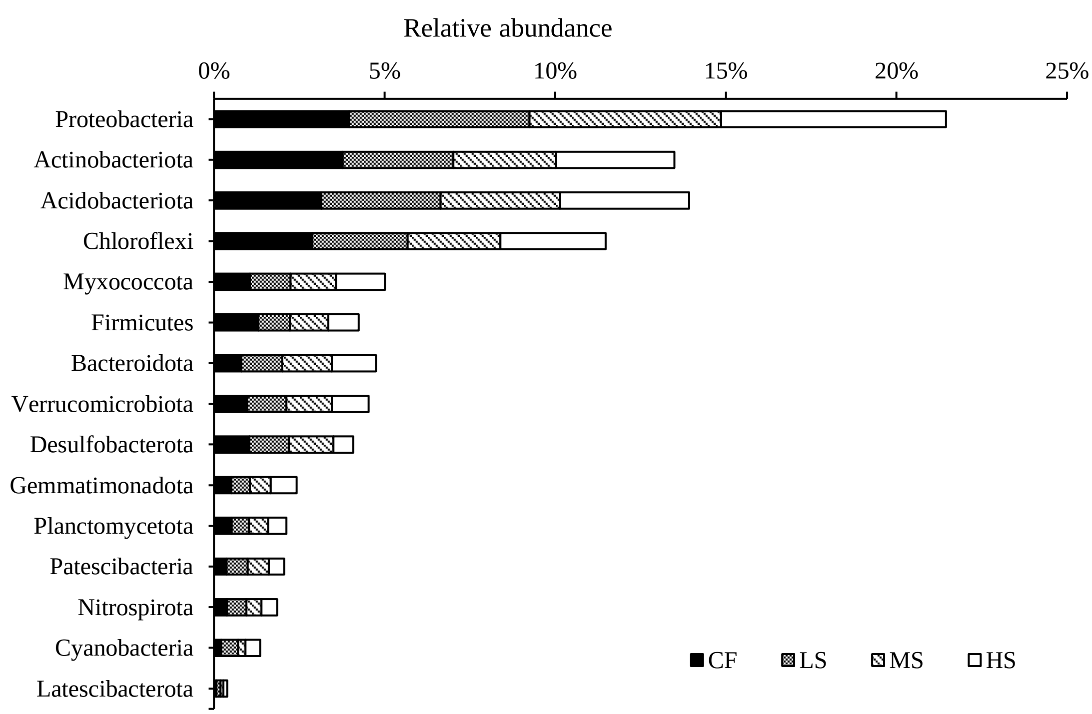

Figure 3.5. Relative abundance of microbial populations in rhizosphere soil at the phylum level.

The three most abundant phyla in all rhizosphere samples collected throughout the growing season were proteobacteria (22.2%), actinobacteriota (14.8%), and acidobacteriota (14.6%), followed by chloroflexi (12.3%) and myxococcota (5.2%). Proteobacteria was present in the highest proportion in the HS treatment, then MS, LS, and CF at 6.6, 5.6, 5.2, and 3.9%, respectively. Actinobacteriota and firmicutes were most abundant in the CF treatment (3.8 and 1.3%, respectively), acidobacteriota, chloroflexi, and myxococcota were most abundant in the HS treatment (3.8, 3.1, and 1.4%, respectively).

At the genus level, the 5 dominant genera belong to the phylum acidobacteriota which represented 11.3% of the total abundance, followed by 4 genera of the phylum actinobacteriota (8.5%). The most abundant was candidatus of the family koriobacter (2.8%) which dominated in the HS treatment (0.7%).

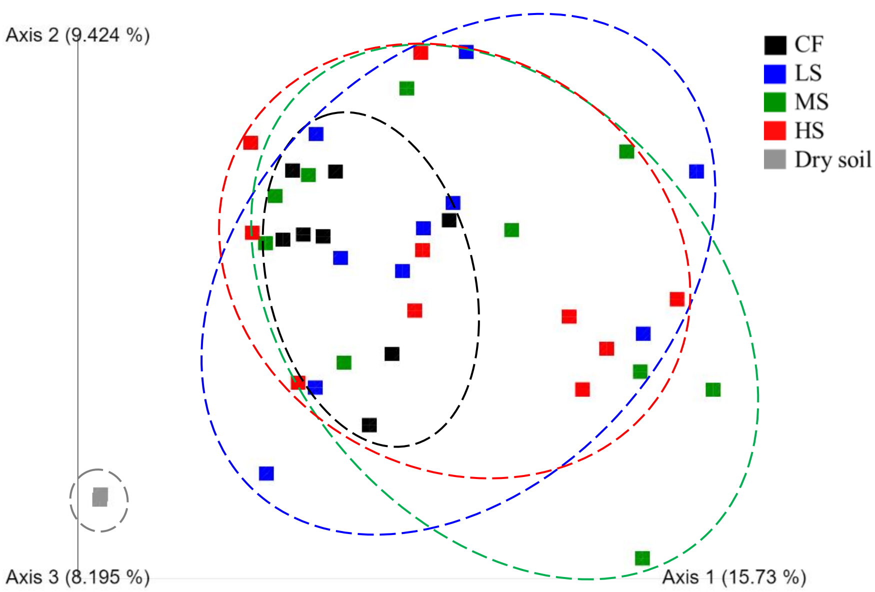

Figure 3.6. Beta diversity Bray-Curtis PCoA analysis by treatment.

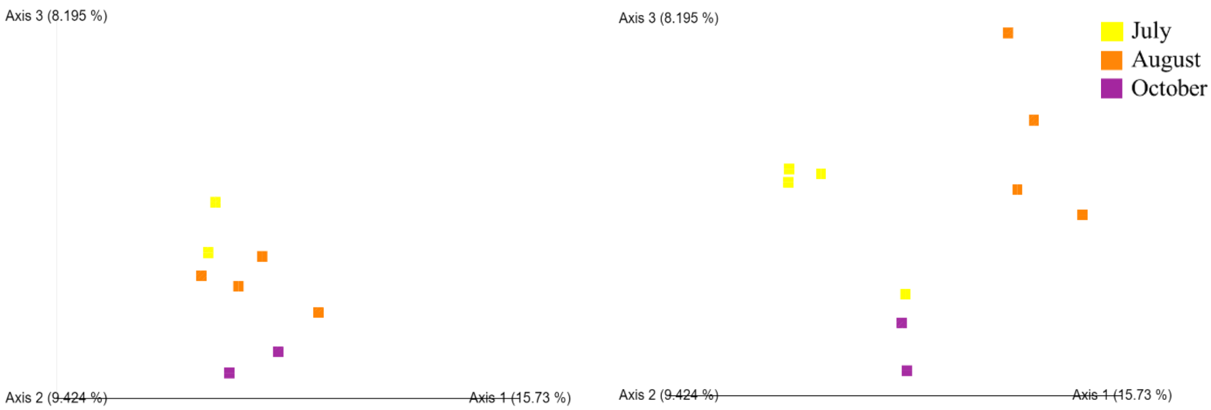

Figure 3.7. Bray-Curtis PCoA analysis for CF (left) and HS (right) treatments by sampling date.

The Bray-Curtis principal coordinates analysis (PCoA) revealed that throughout the growing season, bacterial groups of the CF treatment show statistical similarity based on 3-dimensional factors that influenced 15.73, 9.42, and 8.19% of the variation. Samples of all II treatments show higher variability (Figure 6). When analyzed by sampling date, the highest amount of variation is observed for the August samples. Additionally, we observe a distinction between samples obtained at different dates, which diverge in a larger amount for the HS treatment (Figure 7 and Appendix 3.3).

4. Discussion

Consistent with results presented in Chapter 1, II treatments decreased As concentrations in rice grains, shoots and roots, and increasing dry-down severity had a greater impact in reducing As bioaccumulation (Figure 3 and 4). Like As, the concentration of Fe in root samples with plaque was highest for CF treatment and decreased for LS, MS, and HS treatments (Figure 4). It is surmised that dry-down events (II) promote oxic conditions in the bulk soil and aqueous Fe^{II} is oxidized and immobilized via precipitation of Fe^{III}-oxides, decreasing the overall Fe content in the rhizosphere and root plaque compared to the CF treatment. With CF, mobile aqueous Fe^{II} persists in the bulk soil and is oxidized only after being transported into the oxygenated rhizosphere. Therefore, greater precipitation of Fe^{III}-oxides as root plaque occurs, and a higher total Fe concentration is observed in root samples from the CF treatment.

Soil pH continually increased from sowing to harvest by 1.5 units (mean value for all treatments). This increase is explained by the constant consumption of protons in paddy soils during reduction processes associated with flooded rice paddies (Yu, 1991; Masscheleyn et al., 1991). Soil redox potential (Eh) dropped upon initial flooding, increased above 100 mV during dry-down events, and declined after reflooding below -300 mV for all treatments (Figure 1). This pattern indicates oscillation between anoxic and oxic conditions. The redox state of Fe and As during these fluctuations can be predicted via the Nernst equation or, simply, by utilizing the pH and Eh measurements and referring to Pourbaix diagrams, which indicate that both As and Fe are present in their reduced forms during flooding and become oxidized during the dry-down events of II treatments (Hashimoto and Kanke, 2018; Al-Abed et al., 2007; Masscheleyn et al., 1991).

During the dry-down periods in the three II treatments, soil water potential reached -10, -70, and -120 kPa for LS, MS, and HS treatments, respectively. Based on Carrijo et al., 2018, soil water potentials reached during dry-down events are equivalent to volumetric water contents of 40, 35, and 25% for LS, MS, and HS, respectively. These parameters were carefully defined for the field trials, as well as the timing of the dry-downs, to ensure that rice plants reach maturity and maintain yields (Carrijo et al., 2019).

From 52 identified phyla, the 15 most abundant accounted for 98% of the total sequential reads. The three dominant phyla for all treatments throughout the growing season were proteobacteria, actinobacteriota, and acidobacteriota, which are typically found in agricultural soils (Kuramae et al., 2012; Lopes et al., 2014; Zecchin et al., 2017). Proteobacteria, the most abundant phylum in our soil samples, is expected to thrive in carbon-rich environments, such as rice paddies (Fierer et al., 2012). Although the mentioned phyla predominated in our samples, there were variations in the relative abundance of these groups.

Our samples from the CF treatment express a higher abundance of the phyla actinobacteriota and firmicutes, whereas acidobacteriota, chloroflexi, and myxococcota are more abundant in the HS treatment. These results suggest phylogenetic differences in the bacterial communities are driven by fluctuations of anoxic and oxic conditions. Accordingly, the order anaerolineae of the phylum chloroflexi has been identified as an obligate anaerobe, and the order flavobacteriales of the phylum bacteroidota are aerobic chemoorganotrophs (Lopes et al., 2014). The sequence reads of anaerolineae after dry-down events are highest for the CF treatment, followed by LS. In contrast, flavobacteriales follow the opposite trend, increasing in sequence reads after the dry-down event for HS and MS treatments. Given that the identified aerobic and anaerobic orders in our rhizosphere soil samples correlate with the oxic and anoxic conditions introduced by dry-down treatments and follow an expected trend with regards to the severity of the treatments, we can infer that II treatments of a single dry-down event have the potential to shift the microbial communities of the rhizosphere in rice paddies.

As we aim to understand how changes in the abundance of microbial communities under II treatments throughout a rice growing season affect the chemistry and cycling of elements in the rhizosphere, we recall that in Chapter 2 and in Seyfferth et al., 2017, a positive correlation between As and Fe precipitated in rice root plaque was found. Now we must determine if bacterial communities are contributing to the interactions between these elements in the rhizosphere of rice.

Bacteria of the family geobacteraceae of the phylum desulfobacterota, anaeromyxobacteraceae of the phylum myxococcota (Somenahally et al., 2011; Wang et al., 2019; Zecchin et al., 2017), as well as the genus ferribacterium of the class gammaproteobacteria (Scheid et al., 2004), have been found as iron reducing bacteria in paddy soils. In our study, geobacteraceae was found in a lowest amount in the HS treatment, and anaeromyxobacteraceae in the MS and HS treatments. Both taxa increased in sequence reads between 60-90 days after sowing. Reads of the genus ferribacterium were present in all treatments before dry-down events and decreased progressively to zero following dry-down treatments for HS, MS, and LS.

Additionally, some commonly reported Fe oxidizing bacteria, which may also oxidize As, include genera acidovorax and thiobacillus of the class gammaproteobacteria (Wang et al 2019), and the genus nitrospira of the phylum nitrospirota (Lopes et al., 2014). The sequence reads of thiobacillus were higher for the HS treatment, followed by MS throughout the growing season. Similarly, acidovorax expressed higher reads for the HS treatment after a dry-down event and decreased after reflooding; it was not identified in the CF treatment. Unexpectedly, nitrospira, despite being aerobic, did not follow this trend; higher sequence reads were identified for the LS and CF treatments after dry-down treatments. This genus has been related to different processes involved in Fe, N, and S cycling in the rhizosphere of rice, which could explain a contrasting trend given that it may be impacted by proton exchange processes in these elemental cycles (Lopes et al., 2014).

Our results show changes in aerobic and anerobic, as well as iron oxidizing and reducing, taxa related to II treatments, confirming that oxic and anoxic fluctuations in soil due to a single dry-down event impact the community structure of rice rhizosphere soil bacteria.

Alpha diversity results revealed 20 to 25% more diversity in features within a CF soil sample compared to dry soil before sowing. In contrast, we learned from our PCoA results that the changes in microbial community composition expressed in variability between samples is higher for II treatments than CF throughout the growing season. These changes in the diversity of the II samples are explained by the variability factors such as taxonomic differences and abundance.

Our pot experiment was conducted as a replication of the 2017-2018 rice growing field trials at Biggs, CA explained in Chapter 1. Although water management treatments, as well as plant care were carefully planned, it is necessary to consider that this experiment cannot fully imitate field-conditions in terms of scale, hydrology, and other environmental factors. In fact, it may be considered as a closed system given that each replicate consists of 1 gallon of soil, thus the movement of dissolved constituents of the soil solution is limited, as well as the space for root growth and the balance of air and water in soil (Ogunkunle and Beckett, 1988). In addition, dry-down treatments were not performed in the precise way as field trials, given that our system cannot imitate the evapotranspiration and percolation rates of a paddy field. Instead, bins were drained for 1, 3.5, and 5.5 days for LS, MS, and HS, respectively. Despite having reached the water potential of the II treatments at the field trial, the period of drainage was shorter. Given these differences, we cannot expect that the observed changes in microbial communities from pot trials will necessarily be the same at the field-scale.

Additionally, As concentrations in the soil used for this experiment represented northern California (USA) conditions and are low in comparison to paddy soils in Southeast Asia and other rice growing regions. Consequently, As levels were too low to be the main factor shaping the bacterial communities in this study (Zecchin et al., 2017). It is important to consider that higher concentrations of As may significantly affect the microbial community diversity in paddy soils (Xiong et al., 2010).

5. Conclusions

Intermittent irrigation applied to rice crops is a subject of investigation with increasing interest. It is essential to comprehend the chemical and biological factors at the root-soil interface with different water management treatments to evaluate the process of As uptake into rice. Thus, the primary objective of this study was to evaluate the impacts that II treatments, with a single dry-down period, have on rhizosphere microbial communities and consequently better understand their role in rice As uptake. Our study provides evidence that a single dry-down treatment can decrease As uptake into grain through rhizosphere immobilization that is partially driven by soil microbiota. With the introduction of oxic conditions in II, we identified process specific patterns in bacterial taxa involved in iron precipitation, suggesting a role in As immobilization. Although our results were obtained through a small-scale pot experiment, similar microbial changes are expected to occur at field-scale under these II treatments.

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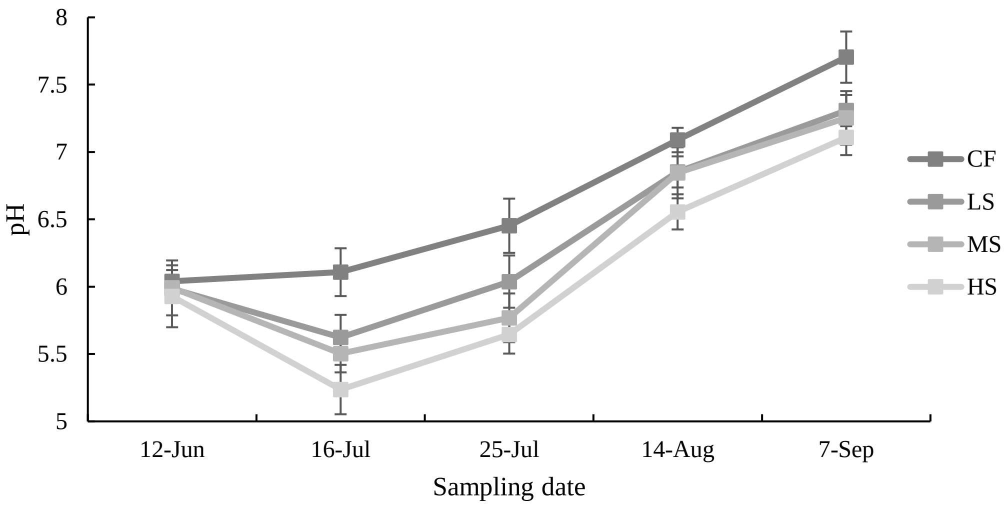
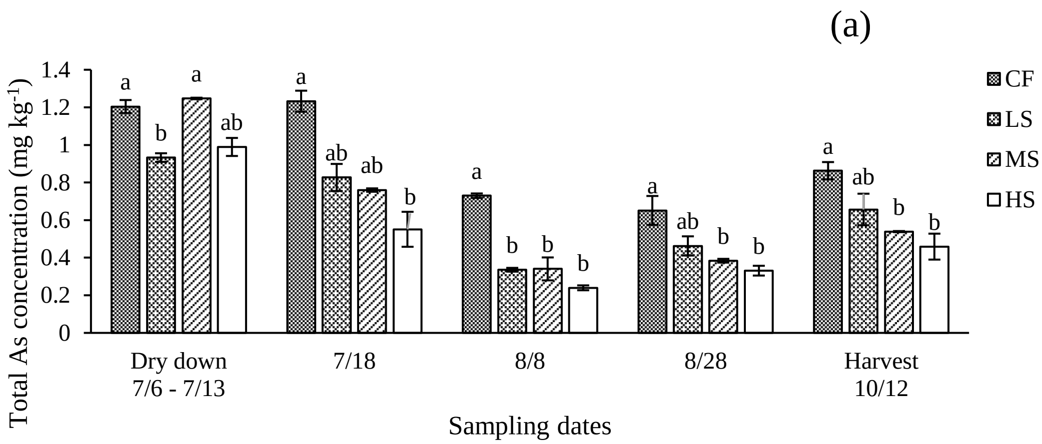
APPENDIX 1. Supplemental information for chapter 1.

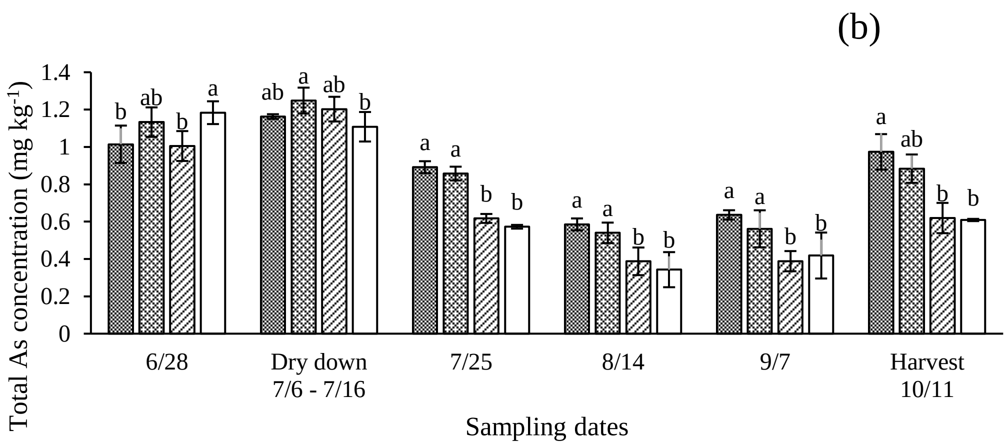
Appendix 1.1



Appendix 1.1. Soil pH during 2018 season.

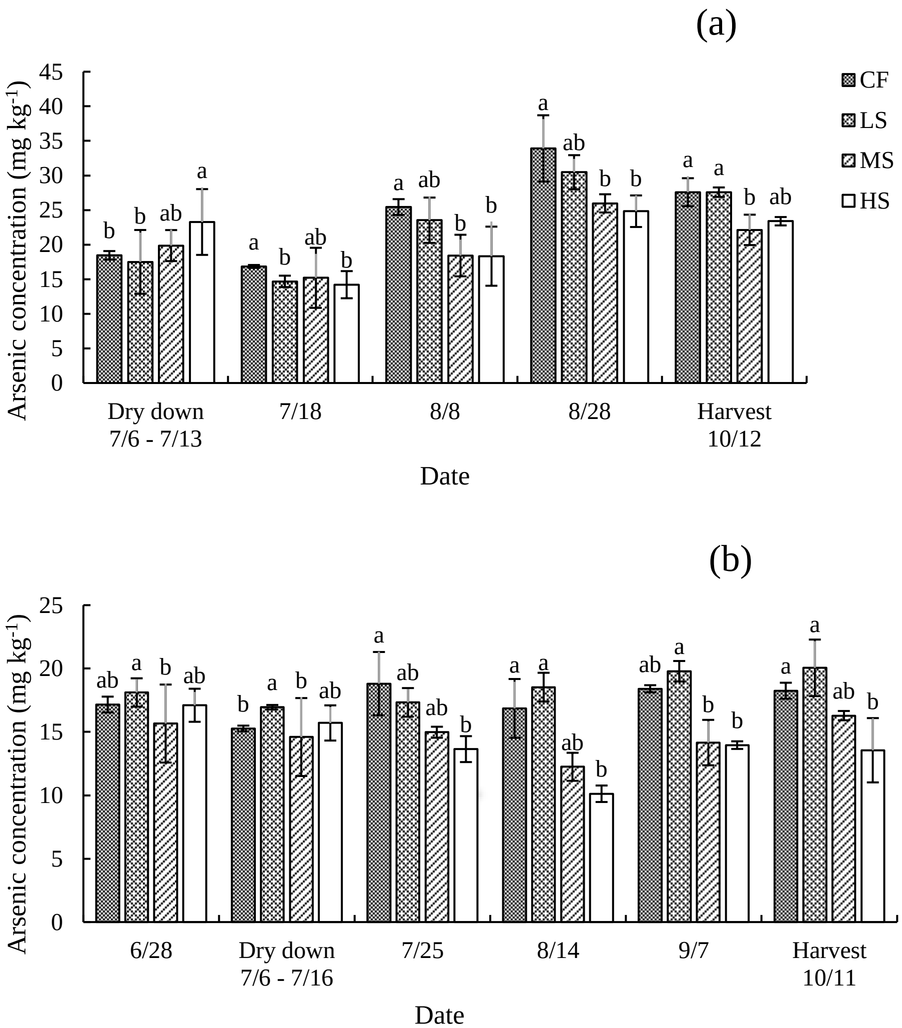
Appendix 1.2





Appendix 1.2. Total arsenic concentrations in shoots throughout the 2017 (a) and 2018 (b) growing seasons. Units in mg of As per kg of plant tissue.

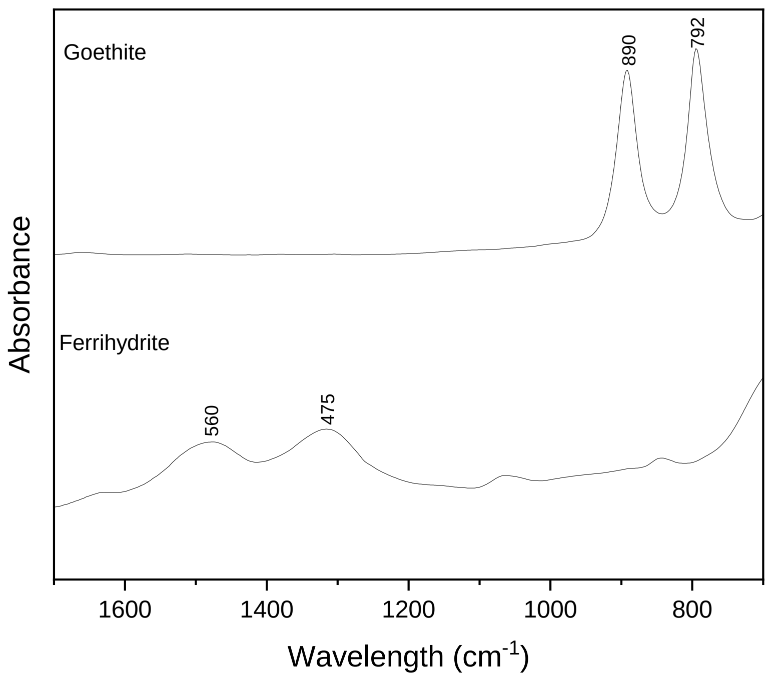
Appendix 1.3



Appendix 1.3. Total arsenic concentrations in roots throughout the 2017 (a) and 2018 (b) growing seasons. Units in mg of As per kg of plant tissue.

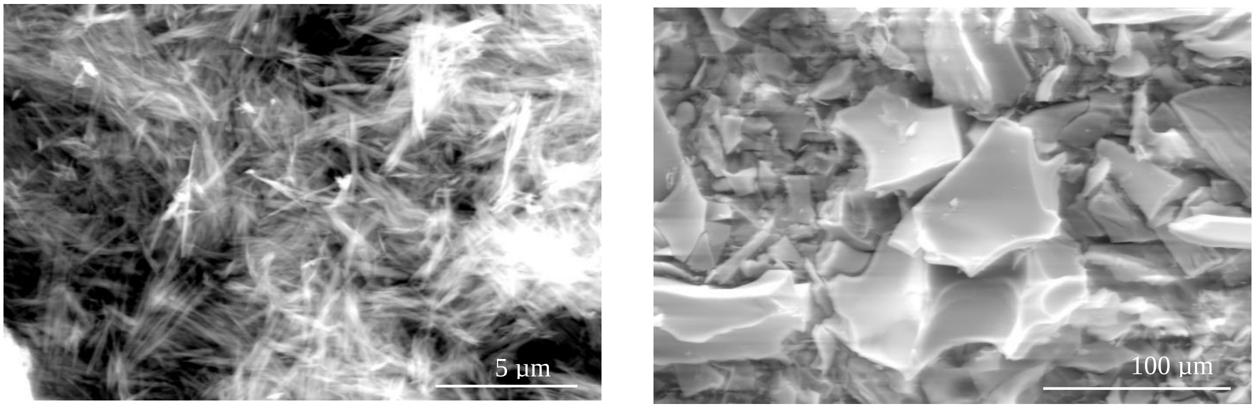
APPENDIX 2. Supplemental information for chapter 2.

Appendix 2.1



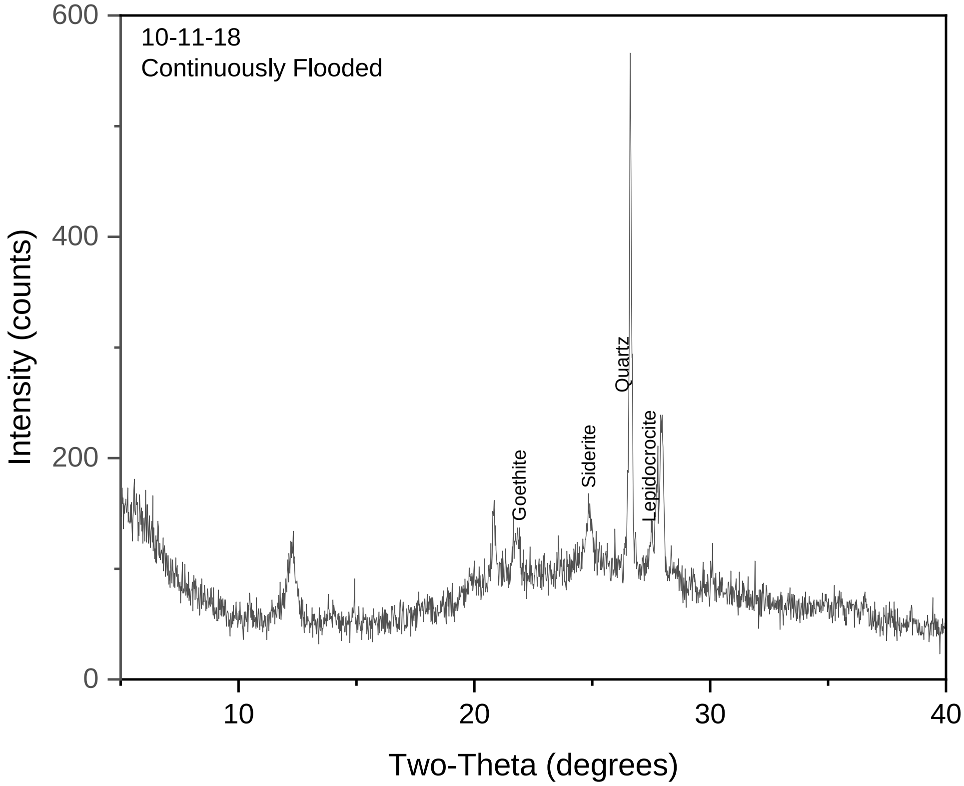
Appendix 2.1. ATR-FTIR spectra confirming the synthesis of goethite and ferrihydrite minerals.

Appendix 2.2



Appendix 2.2. SEM images of synthesized goethite (left) and ferrihydrite (right).

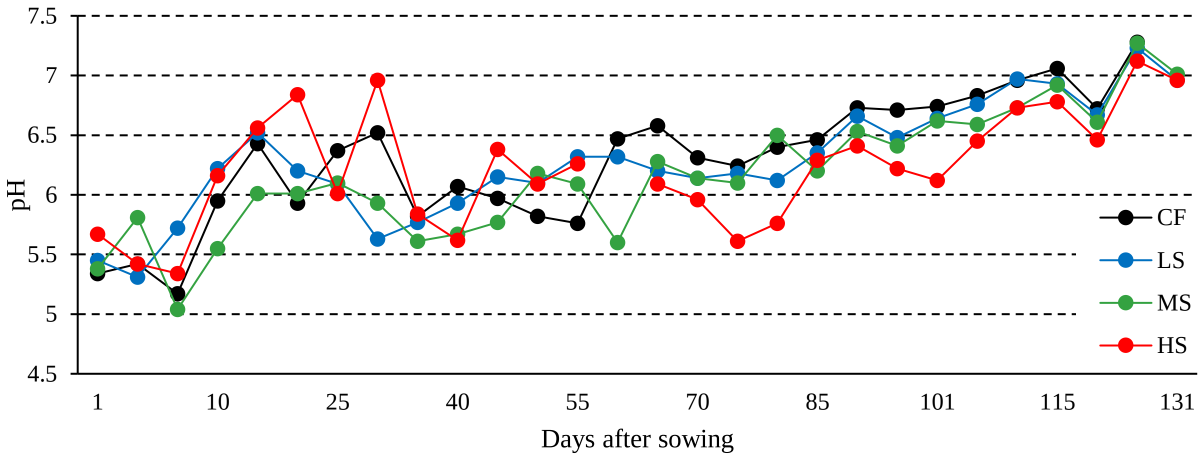
Appendix 2.3



Appendix 2.3. X-ray diffraction pattern of intact root plaque of plants sampled from the continuously flooded (CF) treatment at harvest.

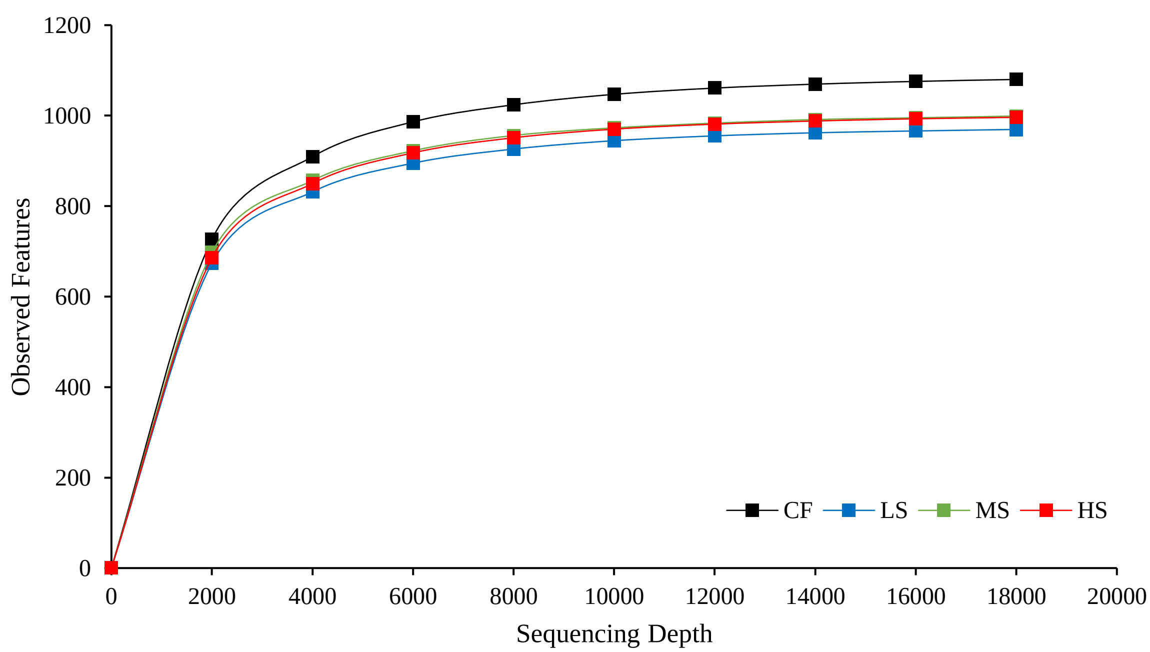
APPENDIX 3. Supplemental information for chapter 3.

Appendix 3.1



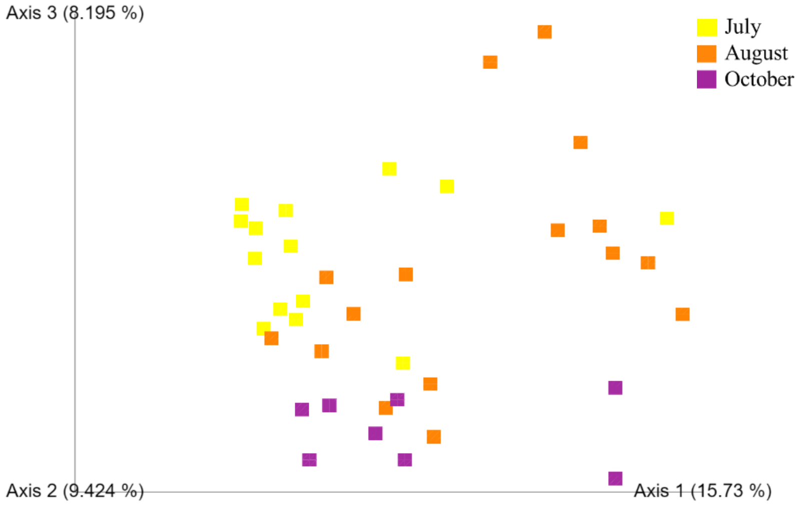
Appendix 3.1. Soil pH throughout the growing season, measured at a 5 cm depth.

Appendix 3.2



Appendix 3.2. Alpha diversity rarefaction, differences in observed features by treatment.

Appendix 3.3



Appendix 3.3. Beta diversity Bray-Curtis PCoA analysis by sampling date.