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Si and Water Management Drives Changes in Fe and Mn Pools that Affect As Cycling and Uptake in Rice

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Abstract: Arsenic availability to rice is tied to biogeochemical cycling of Fe and Mn in rice soils. Two strategies to minimize As uptake by rice—increasing Si and decreasing water—affect soil Fe and Mn pools. We synthesized data from several soil-based experiments with four rice cultivars across pot and field trials with manipulations of Si, water, or both. Increasing Si alters the mineral composition of Fe plaque more than decreasing water, with the former promoting relatively more ferrihydrite and less lepidocrocite. Nonflooded conditions decrease lepidocrocite but slightly increase goethite compared to flooded rice. Plaque As, which was a mixture of arsenite (15–40%) and arsenate (60–85%), was correlated positively with ferrihydrite and negatively with lepidocrocite and goethite. Plaque As was also positively correlated with F1 and F2 soil As, and F2 was correlated positively with porewater As, total grain As, and grain organic As (oAs). Grain inorganic As (iAs) was negatively correlated with oxalate-extractable Fe and Mn. Our data and multiple linear regression models suggest that under flooded conditions iAs is released by poorly crystalline Fe oxides to porewater mainly as iAs(III), which can either be taken up by the plant, adsorbed to Fe plaque, oxidized to iAs(V) or methylated to oAs. Increasing Si can promote more desorption of iAs(III) and promote more poorly-ordered phases in plaque and in bulk soil. The ultimate effectiveness of a Si amendment to decrease As uptake by rice depends upon it being able to increase exogenous Si relative to As in porewater after competitive adsorption/desorption processes. Our data further suggest that poorly crystalline Fe and Mn soil pools can retain inorganic As and decrease plant uptake, but these pools in bulk soil and plaque control grain organic As.

Keywords: rice; Fe plaque; arsenic; silicon; water management

1. Introduction

Arsenic cycling in soils and sediments has implications for food security and human health [1–3]. Arsenic uptake and accumulation by rice may lead to grain sterility and yield reductions [1,4]. Moreover, inorganic As (iAs) is a Class I human carcinogen, so its accumulation in rice grain and bran is detrimental to human health for consumers of rice and rice products [5,6]. Most at-risk populations are individuals with low body mass, those who consume high quantities of rice, and those who also ingest iAs via drinking water [7]. In an effort to devise approaches to minimize As entry into the human food chain, many studies have been conducted to understand how As is released from soils and sediments to water [8–16], where it can be taken up by rice roots. It has become clear that plant-availability of As in rice paddy agroecosystems is intimately tied to biogeochemical Fe and Mn cycling [17,18].

Oxides of Fe and Mn are important for controlling the release and attenuation of As in soils and sediments, including rice paddies. Arsenic adsorbs onto Fe oxides, hydroxides, and oxyhydroxides (hereafter referred to as Fe oxides), but the strength of the interaction depends mainly on the Fe phase, the speciation of As, the pH and Eh of the system. At low pH (pH < \sim 5.5), oxidized iAs (arsenate, As(V), HAsO₄⁻/HAsO₄²⁻) is more able to sorb to Fe oxide phases than reduced iAs (arsenite,

As(III), H₃AsO₃⁰), but as pH increases to 7 or above, relatively more As(III) is able to sorb [19–21]. Organic arsenic (oAs) species in rice paddy soils consist mainly of pentavalent dimethylarsinic acid (DMA), which has much lower affinity for adsorption onto Fe oxides than iAs(V) at pH 4–7, and lesser amounts of pentavalent monomethylarsonic acid (MMA), which has comparable affinity for adsorption to Fe oxides as iAs(V) [22]. In general, poorly crystalline Fe oxide phases are more reactive and have higher surface area, and therefore are able to sorb more As than well-crystalline phases [19,22]. These sorption phenomenon lead to strong retention of As under aerobic or upland conditions, but as soils are flooded, microbially-mediated reductive dissolution of Fe oxides releases adsorbed As. As(III) is more mobile under reducing conditions because unlike As(V), it does not readily sorb to other soil minerals. Mn oxides are not as abundant as Fe oxides and have less capacity for As sorption, but they primarily serve to oxidize As(III) to As(V), which can then be sorbed onto Fe oxides [23].

Fe oxides are abundant in bulk soil and sediment, but also form around paddy rice roots where oxygen transported to roots via aerenchyma leaks out of less suberized roots and aids in Fe(II) oxidation, forming an Fe plaque [24,25]. This Fe plaque can sorb mobilized As [26–29] but may also release As, particularly under wetting and drying cycles [30,31]. As arsenic species move toward roots via transpirational water flux, root uptake of As is slowed down by Fe plaque as As species are adsorbed by Fe plaque. iAs(III) adsorbs as an outer-sphere complex, which is more easily prone to desorption than iAs(V), which adsorbs as an inner sphere complex and thus more tightly bound. Moreover, the Fe plaque coatings on rice roots are discontinuous [32] and are comprised of a mixture of Fe phases [32–36], which change in response to solution chemistry [34–37]. Thus, the ability of Fe oxides in Fe plaque to retain As and lower its uptake into rice depends strongly on the biogeochemical conditions of the rice paddy, which affect the composition and quantity of Fe plaque.

Efforts aimed to lower As mobilization in rice paddies and plant-uptake can impact the interaction of As with oxides of Fe and Mn. For example, increasing porewater Si in rice paddies can lower either total As or iAs uptake and accumulation in rice grain [38,39], but it is well established that increasing solution-phase Si retards the transformation of ferrihydrite to higher ordered phases [40–42]. Amaral et al. [34] showed that as porewater Si increased, the percentage of ferrihydrite, a poorly crystalline Fe phase, in root Fe plaque increased. This stabilization of ferrihydrite in root Fe plaques may provide more surface area for iAs sorption. However, dissolved Si as monomeric silicic acid $(H_4SiO_4^0)$ is chemically similar to iAs(III) and can compete for sorption sites onto Fe oxides [43]. Si-induced desorption of iAs(III) could actually increase plant-available iAs(III) and uptake depending on local conditions [39]. For example, the presence of Mn oxides could oxidize the desorbed iAs(III) to iAs(V) [17,23], or arsenic methylating microbes could respond to the increase in iAs(III) and methylate iAs(III) to MMA and DMA, which have lower affinity for sorption and are therefore more plant-available [44]. Another means of decreasing plant-availability of As in rice paddies is by decreasing the duration of soil flooding [45], which leads to oxidation of iAs(III) to iAs(V) (e.g., via Mn/Fe oxides and/or As oxidizing microbes) and increases iAs(V) retention onto Fe oxides. However, the impact of water management on Fe plaque mineral composition and quantity has not been reported.

Here, we evaluated impacts of water management and Si-rich amendments on changes in pools of As, Fe and Mn in soils, Fe plaque mineral composition and quantity, and how these impacted As speciation and storage in rice grain. We hypothesized that across multiple experiments, soil amendments with available Si would decrease the crystallinity of Fe oxides in root plaques, resulting in higher concentrations of As in root plaques. We report on Fe plaque quantity and mineral composition data collected from across a range of soil-based experiments in both pot and field trials where either Si addition, water management, or both were manipulated and where soil As values ranged from non-contaminated (5 mg/kg) to contaminated (16–25 mg/kg). These data reveal differences in Fe mineral composition and soil Fe and Mn pools with implications for grain As concentrations in rice.

2. Materials and Methods

2.1. Experimentation and Sample Collection

Soil, porewater, Fe plaque, and rice grain data was synthesized from several previously published pot and field studies utilizing four rice (*Oryza sativa* L.) cultivars [34,36,38,46], and we complement these data with additional soil characterization and additional years of data from the field study [36].

2.1.1. Pot Studies

Data were synthesized from three previously published pot studies [34,35,38,46] in which rice was grown in soil amended with different forms of Si under differing soil flooding conditions. Soil contained either background levels of ~5 mg/kg As (Low As, n = 18), this Low As soil spiked with an 80/20 mixture of As(V)/As(III) to achieve ~25 mg/kg As (Spiked As, n = 16), or elevated levels of ~16 mg/kg As (High As, n = 27). Prior to rice growth, soils were amended with one of several Si sources: calcium silicate (Silicate), rice husk (Husk), charred rice husk (Char) or rice husk ash (Ash). Char and Ash differed in that Char was prepared by burning rice husk at a lower temperature than Ash, resulting in less crystallization of the Si [36]. In each experiment, Control soils were left unamended and Si-rich materials were applied at similar Si rates (1% w/w of amendment, equivalent to 5 Mg Si/ha). In all pot studies, soil was flooded conditions. Each treatment was conducted in triplicate. At harvest, the entire root systems were separated from shoots, washed with tap water to remove adhered soil, and rinsed with double deionized water.

2.1.2. Field Studies

The field study was conducted adjacent to where the soil was collected for the Low As pot study (~5 mg/kg As) and used several of the same Si amendments as the pot studies. Twelve paddies received soil silicon amendments including Control, Silicate, Husk, and Char (amended at 5 Mg Si/ha) and each treatment was conducted in triplicate paddies; details of the experimental design are previously published for 2015 [36]. Here we also include two additional years of data for 2016 and 2017 (n = 36). Included in these additional years of data are 6 paddies with unamended soils subjected to varying states of inundation, ranging from flooded to nonflooded (n = 12). Each year at harvest, three plants were collected from a diagonal transect across each 2×2 m paddy and were composited into one sample per paddy. Roots were similarly separated and washed as described in the preceding section.

2.2. Porewater Collection and Analysis

Porewater was collected every 1–2 weeks from each experimental unit using Rhizon samplers inserted into the rooting zone. Porewater was collected using evacuated, sealed headspace vials for the pot studies or by locking syringe for the field studies. Porewater As, Fe and Mn was measured by ICP-MS after acidification to 2% nitric acid. Porewater Si was measured colorimetrically using the molybdenum blue method [47].

2.3. Fe Plaque Characterization

Roots were treated based on previous work to characterize Fe plaque [34]. Briefly, washed and air-dried roots were divided longitudinally: one half was used for dithionite-citrate-bicarbonate (DCB) extraction [48] and total elemental analysis for total As, Cd, Fe, and Si using ICP-OES, and the other half was used for intact Fe plaque sampling via sonication and characterization with bulk XRD and XAS [34]. The half root systems with intact Fe plaque were subject to gentle sonication (2 h at 25 °C) in deionized water and the sonication solution was filtered through nitrocellulose filters to obtain samples of As-bearing Fe plaque minerals on the filters. The filters were placed into a loosely-capped petri dish to air-dry and were transported to Stanford Synchrotron Radiation Lightsource (SSRL) for analyses

with X-ray diffraction (XRD) (beamline 11-3) and analyzed using Match! Software (v3, Crystal Impact, 2015). To quantify the mineral composition of Fe plaques and the oxidation state of As in the Fe plaques, the filtered Fe plaques were characterized with extended X-ray absorption fine structure (Fe EXAFS) spectroscopy (beamlines 4-3 and 11-2) and by X-ray absorption near edge structure (As XANES) for a subset of samples (beamline 11-2). The obtained spectra were background subtracted, normalized, and fitted by linear combination fitting (LCF) using Athena software. The following materials were included as standards: 2-line ferrihydrite (Fe(OH)₃·nH₂O), lepidocrocite (γ -FeOOH), hematite (Fe₂O₃), siderite (FeCO₃), and goethite (α -FeOOH) as Fe standards (constrained by XRD results); and As(III) as NaAsO₂, and As(V) as Na₃AsO₄ as As standards. Note there were no pentavalent DMA, pentavalent MMA, or arsenite glutathione observed in Fe plaques.

2.4. Plant Analysis

Shoots collected at harvest were dried, finely ground, and analyzed following previously published methods [36,38]. Briefly, 0.2–0.5 g of plant tissue was microwave digested in trace metal grade nitric acid. After diluting the sample, the acid fraction was analyzed for total As using ICP-MS. Grain As speciation utilized a 2% HNO₃ extraction [49] followed by separation by HPLC-ICP-MS using a carbonate eluent and a Hamilton PRP-X100 column [50].

2.5. Field Study Soil Fe, Mn, and As Extractions

To characterize the soil pools of As, Fe, and Mn in the field study, targeted extractions were performed on soil collected at harvest in 2015, 2016, 2017. For each paddy, five soil samples were collected at the 0–10 cm depth from two diagonal transects across each paddy and composited into one sample per paddy (n = 48). All collected soils were air dried and passed through a 2-mm sieve for extraction and analysis.

A portion of the 2-mm sieved soil was subjected to As sequential extraction, which uses progressively stronger extractants to target five soil As pools [51]. The extractants in order of weakest to strongest are 0.05 M ammonium sulfate for non-specifically sorbed As (F1 fraction), 0.05 M ammonium phosphate for specifically sorbed As (F2 fraction), 0.2 M acid ammonium oxalate (AAO) for As associated with amorphous and poorly-crystalline Fe/Al oxides (F3 fraction), 0.2 M ammonium oxalate and ascorbic acid for As associated with well-crystalline Fe/Al oxides (F4 fraction), and nitric acid/hydrogen peroxide for residual As phases (F5 fraction). After centrifugation, supernatants were syringe filtered through 0.45 µm filters into acid-washed polypropylene tubes and acidified with 2% nitric acid. Elemental analysis for each extractant, which also included Fe and Mn for F3, was conducted using matrix-matched standards with ICP-MS. Blanks and standard checks were added every 20 samples for QA/QC. NIST 2711 (certified As level 107 ± 5 mg/kg) was included during the last step of As extraction. The recovery for As in the reference soil was $86\% \pm 23\%$. Total reducible Fe and Mn oxides were additionally assessed through citrate-bicarbonate-dithionite (CBD) extraction using standard procedures [52]. All samples were analyzed with inductively coupled plasma-optical emission spectroscopy (ICP-OES). All ICP-OES analyses were conducted using matrix-matched standards and similar QA/QC described for ICP-MS.

2.6. Statistical Analyses

ANOVA was performed using PROC GLM and mean comparisons with the Control were performed using the LSMEANS statement with Dunnett's adjustment to the *p*-value. Regression was performed using PROC REG. Stepwise multiple linear regression for grain and straw As was performed using PROC GLMSELECT. Grain, straw, and porewater As were log transformed to minimize heteroscedasticity. To determine the optimal model, 25% of the data was held out of the model fitting as a validation set and the selected model had the minimum average squared error for the validation set. Squared partial correlation coefficients (partial R²) were calculated using the

PCORR2 option in PROC REG to assess the partial correlation of a descriptor given the presence of other descriptors in the model. All statistics were performed in SAS 9.4.

3. Results

3.1. Influence of Si Amendment on Porewater Si and As Concentrations

Across multiple experiments, Si-rich amendments drove changes in porewater As and Si concentrations (Figure 1). Both Husk and Silicate amendments significantly increased average porewater As concentrations by ~40% relative to Control, whereas the slight increase (~15%) from Char and Ash amendment relative to Control was not significant (Figure 1A). Husk, Char and Silicate treatments increased average porewater Si concentrations significantly by 320%, 165%, and 234%, respectively, relative to Control, but Ash amendment did not (Figure 1B). Therefore, while Husk and Silicate treatments increased porewater As, they simultaneously increased porewater Si by a larger percentage.



Figure 1. Impact of Si amendment on average porewater (SE) (**A**) As and (**B**) Si concentrations across all tested experiments. Asterisks denote treatment is significantly different from Control (p < 0.05).

3.2. Impacts of Si and Flooding Extent on Root Fe Plaque

Amendments with high levels of available Si most affected root plaque Fe mineral composition (Figure 2, p < 0.0001, F = 10). Control plant root plaques, which averaged 44% ferrihydrite, increased to 55–57% ferrihydrite under Husk (p < 0.0001), Char (p = 0.0009), or Silicate (p = 0.0012) amendment. In response to increased ferrihydrite in the root plaques, both lepidocrocite and goethite became proportionally less abundant. The Ash used in these experiments has previously been shown to contain relatively crystalline, insoluble Si [36], resulting in a root plaque Fe mineral composition not significantly different from the Control for all minerals (p > 0.17). Water management slightly affected root plaque Fe mineral composition, with nonflooded root plaques containing proportionally less lepidocrocite. Nonflooded root plaques. The type of study significantly affected root plaque Fe minerals (p < 0.0001, F = 11), largely due to the spiked As study leading to proportionally higher ferrihydrite (Figure 3).

Porewater Si concentrations were positively correlated with root plaque ferrihydrite for all studies (Figure 3). These positive correlations were significant (p < 0.0001, F = 18) and the slopes were not significantly different between study types (p = 0.22, F = 1.5), although the average values of ferrihydrite for each experiment significantly differed (p < 0.0001, F = 12), implying a similar response of ferrihydrite to porewater Si across experiments.



Figure 2. The proportion of major Fe (hydr)oxide mineral phases that comprise Fe plaque on rice roots is affected by both soil Si amendment and water management. For experiments where soil Si was manipulated, asterisks indicate treatments significantly different from the Control for each mineral at the 0.05 level. For experiments where water was manipulated, bars with the same letters are not significantly different at the 0.05 level. Error bars denote the standard error (Control: n = 38; Ash: n = 17; Husk: n = 26; Char: n = 9; Silicate: n = 12; Flooded: n = 87; Nonflooded: n = 15).



Figure 3. Positive relation between average porewater Si concentration and the fraction of ferrihydrite in the root plaques in all tested pot and field experiments. (Spiked As: $R^2 = 0.61$, p = 0.0026, n = 12; High As: $R^2 = 0.15$, p = 0.046, n = 27; Low As: $R^2 = 0.74$, p < 0.0001, n = 15; Low As Field: $R^2 = 0.078$, p = 0.057, n = 47).

3.3. Correlations between Root Plaque As, Plaque Composition, and Soil As Pools

Arsenic in the DCB-extractable root plaque (i.e., plaque As) was strongly, positively correlated to both plaque Fe and plaque Mn (except Spiked As), but was less strongly correlated to plaque Si (Figure 4). Except for the Spiked As study, plaque As was significantly correlated to plaque Fe (p < 0.0001, F = 33–114, R² = 0.57–0.88) and plaque Mn (p < 0.0001, F = 36–92, R² = 0.44–0.85). Plaque Si

was positively correlated with plaque As, and this correlation was significant for the Field studies (p < 0.0001, F = 56, R² = 0.55), but not for the pot studies (p = 0.062-0.59, F = 0.31–1.8, R² = 0.019–0.13). We also explored other potential relationships but found no significant correlations between plaque Fe or plaque Mn and plaque Si (data not shown).



Figure 4. Relations between DCB-extractable As (i.e., plaque As) and (**A**) plaque Si, (**B**) plaque Fe, and (**C**) plaque Mn. Root plaque As was most positively correlated to plaque Fe, but was also positively correlated to plaque Si and Mn for most studies.

Plaque As also correlated with mineral composition of plaque Fe (Figure 5). Root plaques with higher fractions of ferrihydrite contained higher concentrations of As (p < 0.0001, F = 86, R² = 0.46). Conversely, root plaques with higher fractions of lepidocrocite and goethite had lower concentrations of As. Note that this correlation was much stronger for goethite (p < 0.0001, F = 57, R² = 0.36) than for lepidocrocite (p = 0.0027, F = 9.5, R² = 0.087). Additionally, within the High and Low As pot studies a significant positive correlation existed between plaque lepidocrocite and plaque As, suggesting that while the overall trend was that increasing plaque lepidocrocite decreases plaque As, this effect may not be observed in all experiments.



Figure 5. Relations between plaque As and the proportion of Fe plaque comprised of (**A**) ferrihydrite, (**B**) lepidocrocite, and (**C**) goethite across all tested experiments.

As speciation in the Fe plaque measured by XANES revealed only a mixture of arsenite and arsenate that was correlated with other relevant variables. Plaque As was 31% (standard deviation: 6.4%, n = 34) arsenite, with the remainder fit as arsenate. A significant positive correlation was observed between plaque iAs(III) and porewater Fe (Figure 6A), while a significant negative correlation was

observed between plaque iAs(III) and the Mn concentration in the Fe plaque (Figure 6B). Plaque iAs(III) was not well correlated with plaque ferrihydrite ($R^2 = 0.083$, p = 0.11), Si in the Fe plaque ($R^2 = 0.056$, p = 0.18), or porewater Si ($R^2 = 0.070$, p = 0.13).



Figure 6. Relations between plaque iAs(III) and (**A**) porewater Fe and (**B**) Mn concentration in the Fe plaque for selected samples.

In the field study, soil As fractionation was measured to assess relationships between soil As pools and plaque As, revealing significant correlations between root plaque As and the more soluble soil As pools (Figure 7). Plaque As was most strongly correlated with soil As in the F1 fraction (p < 0.0001, F = 26, R² = 0.36), which targets non-specifically sorbed As, followed by soil As in the F2 fraction (p = 0.0023, F = 10, R² = 0.19), which targets specifically sorbed As. Plaque As was not significantly correlated with F3 (p = 0.097, F = 2.9, R² = 0.059, positive correlation) or F4 (p = 0.10, F = 2.8, R² = 0.057, negative correlation) soil As.



Figure 7. Positive relationships between plaque As and soil-extractable As in the (**A**) F1 fraction and (**B**) F2 fraction from the field study.

3.4. Relations between Porewater Si, Oxalate-Extractable Fe in Bulk Soil, and Plant-Available As

In the field study, bulk soil was influenced by variable Si concentrations (Figure 8). Increasing porewater Si was associated with an increase in oxalate-extractable Fe in bulk soil (p = 0.004, F = 14) (Figure 8A). This correlation was weak ($R^2 = 0.24$) and largely driven by four samples (1 husk-amended and 3 silicate-amended paddies) with high porewater Si concentrations in 2017. This increase in oxalate-extractable Fe was associated with a strong and significant increase in the F2

fraction of As in bulk soil (p < 0.0001, F = 61, R² = 0.57) Figure 8B and weaker but significant increase in F1 (R² = 0.24) and F3 (R² = 0.17) soil As (data not shown).



Figure 8. Relations between (**A**) oxalate-extractable Fe and porewater Si and (**B**) F2 soil As and oxalate-extractable Fe from the field study.

3.5. Influence of Soil As Pools on As Porewater Concentrations

Porewater As in the field studies was influenced by various soil As pools. Average porewater As did not depend on F1 (p = 0.51, F = 0.45, R² = 0.0097, Figure 9A), but was positively correlated with F2 and F3 (p < 0.0001, F = 30, R² = 0.39, Figure 9B and p = 0.0058, F = 8.4, R² = 0.15, Figure 9C, respectively). Porewater As was also positively correlated with AAO-extractable Fe (p < 0.0001, F = 19, R² = 0.29), but not CBD-extractable Fe (p = 0.055, F = 3.9, R² = 0.08). Neither AAO-extractable nor CBD-extractable Mn were correlated with porewater As (p > 0.76, F < 0.09, R² < 0.002).



Figure 9. Relations between porewater As and soil-extractable As in the (**A**) F1, (**B**) F2, and (**C**) F3 fractions.

3.6. Correlations between Grain As and Soil As, Fe and Mn Pools

Total concentrations and As species in polished rice grain from the field study was also correlated with more mobile soil As pools. On average, the polished grain contained 0.20 mg/kg total As (standard deviation = 0.14 mg/kg). The high variability in grain As was best explained by F2 (Figure 10A, p < 0.0001, F = 21, R² = 0.31), while other As pools were negligibly correlated with grain As (p > 0.25, F < 1.4, R² < 0.03). Similarly to total grain As, oAs in the polished grain was most correlated to F2 (Figure 10B, p = 0.0025, F = 11, R² = 0.28). Conversely, iAs in the polished grain was not correlated to F2 (p = 0.83, F = 0.05, R² = 0.0018), but was positively correlated to F1 (Figure 10C, p = 0.027, F = 5.5, R² = 0.16) and negatively correlated to F4 (Figure 10D, p = 0.0012, F = 13, R² = 0.32).





Figure 10. Relations between plant-available soil As pools and (**A**) total or (**B**–**D**) speciated grain As in the field study.

In addition to soil As pools, grain As was also correlated with oxalate-extractable pools of Fe and Mn pools in bulk soil (Figure 11). Both total and organic grain As were weakly and positively correlated with oxalate-extractable Fe (respectively Figure 11A, p = 0.0008, F = 12.9, R² = 0.22, and Figure 11B, p = 0.008, F = 8.1, R² = 0.22) but not Mn (p > 0.25, R² < 0.03). In contrast, grain iAs was not correlated with oxalate-extractable Fe (Figure 11C, F = 0.07, p = 0.79, R² = 0.0025) but was weakly and negatively correlated with oxalate-extractable Mn (Figure 11D, p = 0.016, F = 6.6, R² = 0.19).

Multiple linear regression models of grain and straw As across all experiments revealed porewater As was the best predictor of plant As, followed by root plaque constituents and porewater Si. The stepwise linear regression model considered root plaque As, root plaque Fe, root plaque Si, root plaque ferrihydrite, root plaque lepidocrocite, average porewater Fe, average porewater Mn, average porewater Si, and average porewater As, but not soil extractions as these were not available for the pot studies. Of these variables, the optimal model for grain As contained porewater As, plaque As, and plaque Si (p < 0.0001, F = 104, adjusted R² = 0.77, n = 94, Table 1). Grain As was positively correlated with porewater As and plaque As, although the correlation with porewater As was stronger (partial R² = 0.56 and 0.34, respectively, Table 1). Plaque Si was negatively correlated with grain As (partial R² = 0.10). For straw As, the model contained porewater As, porewater Si, and root plaque lepidocrocite (p < 0.0001, F = 220, adjusted R² = 0.87, Table 1). Similar to the grain model, porewater As was most strongly positively correlated with straw As (partial R² = 0.87). Porewater Si was negatively correlated with straw As (partial R² = 0.19).





Figure 11. Relations between concentrations of As in polished rice grain and oxalate-extractable (**A–C**) Fe or (**D**) Mn in bulk soil in the field study.

Log ₁₀ Grain As				Log ₁₀ Straw As			
Parameter	Estimate (SE)	p	Partial R ²	Parameter	Estimate (SE)	p	Partial R ²
log ₁₀ PW As	0.45 (0.042)	< 0.0001	0.56	log ₁₀ PW As	0.98 (0.039)	< 0.0001	0.87
Plaque As	0.0025 (0.00038)	< 0.0001	0.34	PW Si	-0.0010 (0.00021)	< 0.0001	0.19
Plaque Si	-0.00019 (0.000059)	0.0023	0.10	Plaque lepidocrocite	1.3 (0.44)	0.0053	0.08

Table 1. linear regression models for grain and straw arsenic.

4. Discussion

In this study, we sought to understand how changes in rice paddy management affect mineral composition of As-bearing Fe plaque, pools of soil As, Fe and Mn, and how these management-induced biogeochemical changes influence plant availability of As to rice. We synthesized data from a variety of experiments including four different rice cultivars across pot and field studies in which soils were manipulated by either adding Si or altering water availability. As we hypothesized, soil Si amendments leading to the highest porewater Si (Figure 1) favored root plaque comprised of proportionally more ferrihydrite and consequently higher concentrations of As in root plaques (Figures 2–4). While the impact of increasing porewater Si on ferrihydrite stabilization in plaque has been previously reported with smaller data sets [34–36], here we report this phenomenon across studies, which suggests the finding is not an artifact of experimental design or dependent upon rice cultivar. Carefully controlled laboratory studies have illustrated that Si retards the transformation of ferrihydrite to more ordered phases [40,41,53], which supports the increase in ferrihydrite and decrease in lepidocrocite and goethite observed due to Si-rich amendment (Figure 2). Our data also show that Si-rich amendments affected

the mineral composition of Fe plaque on rice roots more so than water management (Figure 2), which to our knowledge has not been previously reported. Nonflooded soil conditions decreased the fraction of lepidocrocite in the root plaques by ~40% while increasing goethite, although the latter was not statistically significant (Figure 2). Because we also observed traces of siderite in the root plaques of nonflooded rice but not flooded rice, we suggest that the higher concentration of CO₂ present in nonflooded rice soils favored siderite and goethite over lepidocrocite [53] in plaque.

While we observed that Si strongly increased the proportion of ferrihydrite in Fe plaque (Figure 3) which is neoformed as Fe(II) and O₂ react in the rhizosphere, impacts of Si on poorly-crystalline Fe pools in bulk soil were less drastic. We used oxalate-extractable Fe as a proxy for poorly-crystalline Fe pools in bulk soil. We observed a significant and positive relation between oxalate-extractable Fe in bulk soil and porewater Si (Figure 8A), but the correlation was weak and was driven mostly by 4 Si-rich paddies. While the impact of Si on the poorly-crystalline Fe oxide pool in bulk soil may have been less than on ferrihydrite in Fe plaque, there was a strong positive correlation between oxalate-extractable Fe and F2 soil As (Figure 8B), which is a proxy for plant-available As because porewater As is most strongly associated with the F2 soil fraction (Figure 9). This finding suggests that addition of Si to paddy soil stabilizes poorly-crystalline Fe oxides in bulk soil, which sorbs As in a moderately plant-available pool. Increasing Si can also increase iAs bioavailability through desorption of iAs from poorly crystalline Fe oxides in bulk soil or in plaque, and could therefore affect plant As levels.

Plant As levels were governed by porewater As and Si, which was driven by changes in pools of As in bulk soil and in plaque. iAs(III) released to porewater by microbially-mediated reductive dissolution of Fe (hydr)oxides and iAs(V) to iAs(III) reduction in bulk flooded soils can be either adsorbed to Fe plaque via outer-sphere complexation, taken up by plant roots via Lsi transporters, oxidized to As(V) by Mn oxides [23], or methylated to organic As (e.g., DMA) by arsenic methylating microorganisms. We observed that both total and organic grain As were positively correlated with F2 soil As and the poorly-crystalline Fe oxide pool (Figures 10 and 11), which are well-correlated predictors (Figure 8B). These relationships indicate poorly-crystalline Fe oxides control As release and organic As formation due to microbially-mediated methylation of iAs(III). Grain iAs, in contrast, was only weakly positively correlated with F1 soil As but negatively correlated with F4 soil As fraction and the oxalate-extractable Mn pool (Figures 10 and 11). Conditions that promoted well-crystalline Fe oxide in bulk soil and stabilization of Mn oxides thus led to less iAs uptake into grain. If more Mn oxides are available, then As(III) oxidation and retention onto Fe oxides may be favored over plant uptake. Conversely, ferrihydrite has been shown to suppress the oxidation of iAs(III) by Mn oxides [54], and therefore a Si-induced increase in ferrihydrite may promote plant-uptake or methylation of iAs(III) over oxidation by Mn oxides. Owing to the higher adsorption capacity of poorly-crystalline Fe phases like ferrihydrite than well-crystalline phases [55], Fe plaques with more ferrihydrite contained higher levels of associated As (Figure 5). This adsorbed As was present mainly as iAs(V) (60–85%) due to oxidation of iAs(III) either upon adsorption or by Mn oxides prior to adsorption onto the Fe plaque, although more reduced conditions (i.e., higher porewater Fe) led to higher iAs(III) (Figure 6A). Mn concentration in the Fe plaque was negatively correlated with iAs(III), suggesting Mn in the plaque was able to oxidize iAs(III) (Figure 6B). Still, plaque contained a sizeable portion of iAs(III) (15–40%) that could be desorbed and become plant-available. Thus, while iAs adsorption onto Fe plaque undoubtedly slows As uptake by roots, the strong positive relation between plaque As and plant-available As pools F1 and F2 (Figures 7 and 9) could also indicate that plaque As is a source of As to rice. Several hydroponic studies have shown that Fe plaque increases As(III) uptake, but not As(V) uptake [26,56]. The positive relation between plaque As and grain As in the multiple regression model (Table 1) supports plaque contributing a source of As to rice; however, the porewater As source is larger. While plaque As is correlated to F1 and F2 soil As (Figure 7), porewater As is correlated to F2 and F3 soil As (Figure 9), suggesting these two measures of plant-available As differ in their

origin. Likely, the relative amounts of porewater and/or plaque Si and As play a role in the ultimate concentrations of plant As.

Amendments that led to high Si relative to As in plaque and porewater restricted plant As levels, and these ratios are dynamic. Results from the multiple linear regression models indicated that porewater and plaque As and/or Si levels explained most of the variation in grain and straw As with porewater or plaque As showing a direct relation and porewater or plaque Si showing an inverse relation with plant As. The mechanism is due to multiple processes. First, regardless of whether a Si amendment increases porewater As relative to Control (Figure 1A), its ability to also provide sufficient Si to porewater after competitive desorption of arsenite in bulk soil (Figure 1B) allows for Si to compete with As for root-uptake via the highly-efficient Si/As root transport pathway [57,58] in rice. Second, higher exogenous Si leads to decreased gene expression of Si root transporters [59–61], thus restricting the potential pathways (i.e., Si/As root transporters) for As uptake. Third, higher exogenous Si promotes ferrihydrite-rich plaque (Figure 3 and ref. [34]), which helps to slow down As delivery to the root transporters. However, this plaque As could be exchangeable—particularly if comprised of iAs(III) which forms outer sphere complexes, and thus is prone to desorption and plant uptake at grain filling. Likely, higher Si leads to more iAs(III) desorption both from bulk soil and from plaque, and lower plant As levels would be seen where conditions favored relatively high exogenous Si after accounting for competitive desorption between Si and iAs(III). Straw As levels reflect conditions of these processes over a majority of the ~4-month growth cycle of rice, whereas grain levels are more reflective of rhizosphere conditions during reproduction. For example, Teasley et al. [35] showed that while both Silicate and Husk amendments increased porewater Si, the differences in Si solubility between the amendments resulted in Husk being better able to provide porewater Si at grain filling to compete with As for uptake and grain storage and therefore resulted in higher grain yield. Thus, while estimates of plant-available Si and As provide snapshots of conditions at harvest, it is important to consider the dynamic nature of the rhizosphere conditions over the growth cycle [31,62]. Thus, although some Si amendments led to an increase in porewater, plaque, or exchangeable soil (i.e., plant-available) As, their ability to also increase exogenous Si to compete with iAs(III) for uptake after competitive adsorption/desorption processes on the solid phase will dictate plant levels.

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References

- Duxbury, J.M.; Mayer, A.B.; Lauren, J.G.; Hassan, N. Food chain aspects of arsenic contamination in Bangladesh: Effects on quality and productivity of rice. *J. Environ. Sci. Health Part A* 2003, *38*, 61–69. [CrossRef]
- 2. Meharg, A.A. Arsenic in rice-understanding a new disaster for South-East Asia. *Trends Plant Sci.* 2004, *9*, 415–417. [CrossRef] [PubMed]
- 3. Zhao, F.J.; McGrath, S.P.; Meharg, A.A. Arsenic as a food chain contaminant: Mechanisms of plant uptake and metabolism and mitigation strategies. *Annu. Rev. Plant Biol.* **2010**, *61*, 535–559. [CrossRef]
- 4. Panaullah, G.M.; Alam, T.; Hossain, M.B.; Loeppert, R.H.; Lauren, J.G.; Meisner, C.A.; Ahmed, Z.U.; Duxbury, J.M. Arsenic toxicity to rice (Oryza sativa L.) in Bangladesh. *Plant Soil* **2009**, *317*, 31–39. [CrossRef]

- Meharg, A.A.; Deacon, C.; Campbell, R.C.; Carey, A.M.; Williams, P.N.; Feldmann, J.; Raab, A. Inorganic arsenic levels in rice milk exceed EU and US drinking water standards. *J. Environ. Monit.* 2008, 10, 428–431. [CrossRef] [PubMed]
- 6. Meharg, A.A.; Sun, G.; Williams, P.N.; Adomako, E.; Deacon, C.; Zhu, Y.G.; Feldmann, J.; Raab, A. Inorganic arsenic levels in baby rice are of concern. *Environ. Pollut.* **2008**, 152, 746–749. [CrossRef] [PubMed]
- Meharg, A.A.; Raab, A. Getting to the bottom of arsenic standards and guidelines. *Environ. Sci. Technol.* 2010, 44, 4395–4399. [CrossRef] [PubMed]
- 8. Polizzotto, M.L.; Kocar, B.D.; Benner, S.G.; Sampson, M.; Fendorf, S. Near-surface wetland sediments as a source of arsenic release to ground water in Asia. *Nature* **2008**, *454*, 505–508. [CrossRef]
- 9. Fendorf, S.; Michael, H.A.; Geen, A.V. Spatial and Temporal Variations of Groundwater Arsenic in South and Southeast Asia. *Science* 2010, *328*, 1123–1127. [CrossRef] [PubMed]
- Erban, L.E.; Gorelick, S.M.; Zebker, H.A.; Fendorf, S. Release of arsenic to deep groundwater in the Mekong Delta, Vietnam, linked to pumping-induced land subsidence. *Proc. Natl. Acad. Sci. USA* 2013, 110, 13751–13756. [CrossRef]
- Schaefer, M.V.; Ying, S.C.; Benner, S.G.; Duan, Y.; Wang, Y.; Fendorf, S. Aquifer Arsenic Cycling Induced by Seasonal Hydrologic Changes within the Yangtze River Basin. *Environ. Sci. Technol.* 2016, 50, 3521–3529. [CrossRef] [PubMed]
- Schaefer, M.V.; Guo, X.; Gan, Y.; Benner, S.G.; Griffin, A.M.; Gorski, C.A.; Wang, Y.; Fendorf, S. Redox controls on arsenic enrichment and release from aquifer sediments in central Yangtze River Basin. *Geochim. Cosmochim. Acta* 2017, 204, 104–119. [CrossRef]
- Ying, S.C.; Schaefer, M.V.; Cock-Esteb, A.; Li, J.; Fendorf, S. Depth Stratification Leads to Distinct Zones of Manganese and Arsenic Contaminated Groundwater. *Environ. Sci. Technol.* 2017, *51*, 8926–8932. [CrossRef] [PubMed]
- Gillispie, E.C.; Andujar, E.; Polizzotto, M.L. Chemical controls on abiotic and biotic release of geogenic arsenic from Pleistocene aquifer sediments to groundwater. *Environ. Sci. Process. Impacts* 2016, *18*, 1090–1103. [CrossRef] [PubMed]
- 15. Gillispie, E.C.; Matteson, A.R.; Duckworth, O.W.; Neumann, R.B.; Phen, N.; Polizzotto, M.L. Chemical variability of sediment and groundwater in a Pleistocene aquifer of Cambodia: Implications for arsenic pollution potential. *Geochim. Cosmochim. Acta* **2019**, *245*, 441–458. [CrossRef]
- Williams, P.N.; Zhang, H.; Davison, W.; Meharg, A.A.; Hossain, M.; Norton, G.J.; Brammer, H.; Islam, M.R. Organic matter-solid phase interactions are critical for predicting arsenic release and plant uptake in Bangladesh paddy soils. *Environ. Sci. Technol.* 2011, 45, 6080–6087. [CrossRef] [PubMed]
- 17. Xu, X.; Chen, C.; Wang, P.; Kretzschmar, R.; Zhao, F.J. Control of arsenic mobilization in paddy soils by manganese and iron oxides. *Environ. Pollut.* **2017**, *231*, 37–47. [CrossRef] [PubMed]
- 18. Hossain, M.B.; Jahiruddin, M.; Panaullah, G.M.; Loeppert, R.H.; Islam, M.R.; Duxbury, J.M. Spatial variability of arsenic concentration in soils and plants, and its relationship with iron, manganese and phosphorus. *Environ. Pollut.* **2008**, 156, 739–744. [CrossRef] [PubMed]
- 19. Dixit, S.; Hering, J.G. Comparison of arsenic(V) and arsenic(III) sorption onto iron oxide minerals: Implications for arsenic mobility. *Environ. Sci. Technol.* **2003**, *37*, 4182–4189. [CrossRef]
- 20. Goldberg, S.; Johnston, C.T. Mechanisms of arsenic adsorption on amorphous oxides evaluated using macroscopic measurements, vibrational spectroscopy, and surface complexation modeling. *J. Colloid Interface Sci.* 2001, 234, 204–216. [CrossRef]
- 21. Jain, A.; Raven, K.P.; Loeppert, R.H. Arsenite and arsenate adsorption on ferrihydrite: Surface charge reduction and net OH- release stoichiometry. *Environ. Sci. Technol.* **1999**, *33*, 1179–1184. [CrossRef]
- 22. Lafferty, B.J.; Loeppert, R.H. Methyl arsenic adsorption and desorption behavior on iron oxides. *Environ. Sci. Technol.* **2005**, *39*, 2120–2127. [CrossRef] [PubMed]
- 23. Ying, S.C.; Kocar, B.D.; Fendorf, S. Oxidation and competitive retention of arsenic between iron- and manganese oxides. *Geochim. Cosmochim. Acta* 2012, *96*, 294–303. [CrossRef]
- 24. Armstrong, W. Oxidising activity of roots in waterlogged soils. Physiol. Plant. 1967, 20, 920–926. [CrossRef]
- 25. Chen, C.C.; Dixon, J.B.; Turner, F.T. Iron coatings on rice roots-Morphology and models of development. *Soil Sci. Soc. Am. J.* **1980**, *44*, 1113–1119. [CrossRef]

- Chen, Z.; Zhu, Y.G.; Liu, W.J.; Meharg, A.A. Direct evidence showing the effect of root surface iron plaque on arsenite and arsenate uptake into rice (Oryza sativa) roots. *New Phytol.* 2005, 165, 91–97. [CrossRef] [PubMed]
- Hu, Y.; Li, J.H.; Zhu, Y.G.; Huang, Y.Z.; Hu, H.Q.; Christie, P. Sequestration of As by iron plaque on the roots of three rice (Oryza sativa L.) cultivars in a low-P soil with or without P fertilizer. *Environ. Geochem. Health* 2005, 27, 169–176. [CrossRef]
- Liu, W.J.; Zhu, Y.G.; Smith, F.A. Effects of iron and manganese plaques on arsenic uptake by rice seedlings (Oryza sativa L.) grown in solution culture supplied with arsenate and arsenite. *Plant Soil* 2005, 227, 127–138. [CrossRef]
- 29. Liu, W.J.; Zhu, Y.G.; Hu, Y.; Williams, P.N.; Gault, A.G.; Meharg, A.A.; Charnock, J.M.; Smith, F.A. Arsenic sequestration in iron plaque, its accumulation and speciation in mature rice plants (Oryza sativa L.). *Environ. Sci. Technol.* **2006**, *40*, 5730–5736. [CrossRef]
- 30. Yamaguchi, N.; Nakamura, T.; Dong, D.; Takahashi, Y.; Amachi, S.; Makino, T. Arsenic release from flooded paddy soils is influenced by speciation, Eh, pH, and iron dissolution. *Chemosphere* **2011**, *83*, 925–932. [CrossRef]
- Yamaguchi, N.; Ohkura, T.; Takahashi, Y.; Maejima, Y.; Arao, T. Arsenic Distribution and Speciation near Rice Roots Influenced by Iron Plaques and Redox Conditions of the Soil Matrix. *Environ. Sci. Technol.* 2014, 48, 1549–1556. [CrossRef] [PubMed]
- 32. Seyfferth, A.L.; Webb, S.M.; Andrews, J.C.; Fendorf, S. Arsenic localization, speciation, and co-occurrence with iron on rice (Oryza sativa L.) roots having variable Fe coatings. *Environ. Sci. Technol.* **2010**, *44*, 8108–8113. [PubMed]
- Seyfferth, A.L.; Webb, S.M.; Andrews, J.C.; Fendorf, S. Defining the distribution of arsenic species and plant nutrients in rice (Oryza sativa L.) from the root to the grain. *Geochim. Cosmochim. Acta* 2011, 75, 6655–6671. [CrossRef]
- Amaral, D.C.; Lopes, G.; Guilherme, L.R.; Seyfferth, A.L. A new approach to sampling lintact Fe plaque reveals Si-induced changes in Fe mineral composition and shoot As in rice. *Environ. Sci. Technol.* 2017, 51, 38–45. [CrossRef] [PubMed]
- 35. Teasley, W.A.; Limmer, M.A.; Seyfferth, A.L. How rice (Oryza sativa L) responds to elevated As under different Si-rich soil amendments. *Environ. Sci. Technol.* **2017**, *51*, 10335–10343. [CrossRef]
- 36. Limmer, M.A.; Mann, J.; Amaral, D.C.; Vargas, R.; Seyfferth, A.L. Silicon-rich amendments in rice paddies: Effects on arsenic uptake and biogeochemistry. *Sci. Total Environ.* **2018**, *624*, 1360–1368. [CrossRef]
- 37. Seyfferth, A.L. Abiotic effects of dissolved oxyanions on iron plaque quantity and mineral composition in a simulated rhizosphere. *Plant Soil* **2015**, *397*, 43–61. [CrossRef]
- Seyfferth, A.L.; Morris, A.H.; Gill, R.; Kearns, K.A.; Mann, J.N.; Paukett, M.; Leskanic, C. Soil incorporation of silica-rich rice husk decreases inorganic arsenic in rice grain. *J. Agric. Food Chem.* 2016, 64, 3760–3766. [CrossRef]
- 39. Seyfferth, A.L.; Fendorf, S. Silicate mineral impacts on the uptake and storage of arsenic and plant nutrients in rice (Oryza sativa L.). *Environ. Sci. Technol.* **2012**, *46*, 13176–13183. [CrossRef]
- 40. Schwertmann, U.; Taylor, R.M. Influence of silicate on transformation of lepidocrocite to goethite. *Clays Clay Miner.* **1972**, *20*, 159–164. [CrossRef]
- 41. Schwertmann, U.; Thalmann, H. Influence of Fe (II), Si, and pH on formation of lepidocrocite and ferrihydrite during oxidation of aqueous FeCl₂ solutions. *Clay Miner.* **1976**, *11*, 189–200. [CrossRef]
- 42. Anderson, P.R.; Benjamin, M.M. Effects of silicon on the crystallization and adsorption properties of ferric oxides. *Environ. Sci. Technol.* **1985**, *19*, 1048–1053. [CrossRef] [PubMed]
- 43. Luxton, T.P.; Tadanier, C.J.; Eick, M.J. Mobilization of arsenite by competitive interaction with silicic acid. *Soil Sci. Soc. Am. J.* **2006**, *70*, 204–214. [CrossRef]
- 44. Seyfferth, A.L.; Limmer, M.A.; Dykes, G.E. On the use of silicon as an agronomic mitigation strategy to decrease arsenic uptake by rice. *Adv. Agron.* **2018**, *149*, 49–91.
- 45. Xu, X.Y.; McGrath, S.P.; Meharg, A.A.; Zhao, F.J. Growing rice aerobically markedly decreases arsenic accumulation. *Environ. Sci. Technol.* **2008**, *42*, 5574–5579. [CrossRef] [PubMed]
- 46. Seyfferth, A.L.; Amaral, D.; Limmer, M.A.; Guilherme, L.R. Combined impacts of Si-rich rice residues and flooding extent on grain As and Cd in rice. *Environ. Int.* **2019**, *128*, 301–309. [CrossRef] [PubMed]

- 47. Kraska, J.E.; Breitenbeck, G.A. Simple, Robust Method for Quantifying Silicon in Plant Tissue. *Commun. Soil Sci. Plant Anal.* **2010**, *41*, 2075–2085. [CrossRef]
- 48. Taylor, G.J.; Crowder, A.A. Use of the DCB technique for extraction of hydrous iron oxides from roots of wetland plants. *Am. J. Bot.* **1983**, *70*, 1254–1257. [CrossRef]
- Maher, W.; Foster, S.; Krikowa, F.; Donner, E.; Lombi, E. Measurement of inorganic arsenic species in rice after nitric acid extraction by HPLC-ICPMS: Verification using XANES. *Environ. Sci. Technol.* 2013, 47, 5821–5827. [CrossRef] [PubMed]
- 50. Jackson, B.P. Fast ion chromatography-ICP-QQQ for arsenic speciation. *J. Anal. At. Spectrom.* **2015**, *30*, 1405–1407. [CrossRef] [PubMed]
- 51. Wenzel, W.W.; Kirchbaumer, N.; Prohaska, T.; Stingeder, G.; Lombi, E.; Adriano, D.C. Arsenic fractionation in soils using an improved sequential extraction procedure. *Anal. Chim. Acta* **2001**, *436*, 309–323. [CrossRef]
- 52. Sparks, D.L. *Methods of Soil Analysis. Part 3, Chemical Methods*; Soil Science Society of America: Madison, WI, USA, 1996.
- 53. Cornell, M.R.; Schwertmann, U. *The Iron Oxides: Structure, Properties, Reactions, Occurrences, and Uses,* 2nd ed.; Wiley-VCH: Weinheim, Germany, 2003.
- 54. Ehlert, K.; Mikutta, C.; Kretzschmar, R. Impact of Birnessite on Arsenic and Iron Speciation during Microbial Reduction of Arsenic-Bearing Ferrihydrite. *Environ. Sci. Technol.* **2014**, *48*, 11320–11329. [CrossRef] [PubMed]
- Ona-Nguema, G.; Morin, G.; Juillot, F.; Calas, G.; Brown, G.E. EXAFS analysis of arsenite adsorption onto two-line ferrihydrite, hematite, goethite, and lepidocrocite. *Environ. Sci. Technol.* 2005, *39*, 9147–9155. [CrossRef] [PubMed]
- 56. Deng, D.; Wu, S.C.; Wu, F.Y.; Deng, H.; Wong, M.H. Effects of root anatomy and Fe plaque on arsenic uptake by rice seedlings grown in solution culture. *Environ. Pollut.* **2010**, *158*, 2589–2595. [CrossRef] [PubMed]
- 57. Ma, J.F.; Yamaji, N. A cooperative system of silicon transport in plants. *Trends Plant Sci.* **2015**, *20*, 435–442. [CrossRef] [PubMed]
- Ma, J.F.; Yamaji, N.; Mitani, N.; Xu, X.Y.; Su, Y.H.; McGrath, S.P.; Zhao, F.J. Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. *Proc. Natl. Acad. Sci. USA* 2008, 105, 9931–9935. [CrossRef] [PubMed]
- 59. Limmer, M.A.; Wise, P.; Dykes, G.E.; Seyfferth, A.L. Silicon decreases dimethylarsinic acid concentration in rice grain and mitigates straighthead disorder. *Environ. Sci. Technol.* **2018**, *52*, 4809–4816. [CrossRef]
- 60. Ma, J.F.; Tamai, K.; Yamaji, N.; Mitani, N.; Konishi, S.; Katsuhara, M.; Ishiguro, M.; Murata, Y.; Yano, M. A silicon transporter in rice. *Nature* **2006**, *440*, 688–691. [CrossRef]
- 61. Ma, J.F.; Yamaji, N.; Mitani, N.; Tamai, K.; Konishi, S.; Fujiwara, T.; Katsuhara, M.; Yano, M. An efflux transporter of silicon in rice. *Nature* **2007**, *448*, 209. [CrossRef]
- 62. Garnier, J.M.; Travassac, F.; Lenoble, V.; Rose, J.; Zheng, Y.; Hossain, M.S.; Chowdhury, S.H.; Biswas, A.K.; Ahmed, K.M.; Cheng, Z.; et al. Temporal variations in arsenic uptake by rice plants in Bangladesh: The role of iron plaque in paddy fields irrigated with groundwater. *Sci. Total Environ.* **2010**, *408*, 4185–4193. [CrossRef]



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